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PHILIPPINE TOTAQUINA [1]

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TWO TEXT FIGURES

GENERAL INTRODUCTION

Totaquina is the name suggested in 1931 by the subcommittee of experts of the Health Organization of the League of Nations (1) for a new standard preparation of the total alkaloids of cinchona bark. This product is recommended as a cheap remedy for malaria in places where the cost of quinine is prohibitive. Our paper reports some clinical results following the use of totaquina extracted at the Bureau of Science from cinchona bark of trees grown by the Bureau of Forestry in Bukidnon, Mindanao. In other words, the drug discussed in this paper is

[1] These studies were carried out with the coöperation of the Bureaus of Science, Prisons, and Forestry, together with Malaria Investigations, which is jointly supported by the Bureau of Science and the International Health Division of the Rockefeller Foundation. The cinchona bark was supplied by the Bureau of Forestry through the courtesy of Director A. F. Fischer. Totaquina was extracted at the Bureau of Science and was tested clinically at the Iwahig Penal Colony of the Bureau of Prisons, with the assistance of the staff of Malaria Investigations.

The writers desire to express their thanks to Dr. Mariano V. del Rosario, director of the School of Pharmacy, University of the Philippines, for the use of a drug mill in powdering the cinchona bark. Also to Forester Agapito L. Cenabre for information regarding cinchona bark in the Philippines.

entirely a Philippine product, produced in experimental quantities. This paper, therefore, suggests a new and potentially important local industry which might have far-reaching effects in combating malaria in the Islands.

One of us(2) has estimated that there are probably at least 2,000,000 cases of malaria a year in the Philippines. Assuming that the average quinine requirement per case is 250 grains (a low figure), it was stated that the Islands could theoretically use about 32,400 kilograms. Actually, the annual importation of quinine is frequently less than 2,000 kilograms (Table 1).

TABLE 1.—*Quantity of quinine imported into the Philippine Islands, 1898–1932.*[a]

Year.	Amount.	Year.	Amount.	Year.	Amount.
	Hecto-grams.		*Hecto-grams.*		*Hecto-grams.*
1899	[b] 1,489	1911	17,905	1923	13,143
1900	[b] 10,515	1912	21,811	1924	15,029
1901	[b] 8,227	1913	24,352	1925	17,422
1902	[b] 7,226	1914	23,712	1926	22,522
1903	[b] 5,251	1915	23,127	1927	19,028
1904	[b] 7,724	1916	90,731	1928	[d] 28,375
1905	[b] 11,857	1917	27,703	1929	[d] 21,442
1906	[b] 12,865	1918	76,343	1930	[d] 23,410
1907	[b] 10,841	1919	87,511	1931	[d] 20,662
1908	[c]	1920	40,703	1932	[d] 14,839
1909	[c]	1921	7,892		
1910	[c]	1922	8,560		

[a] This table is taken from Russell.(2) The figures, supplied by the Bureau of Customs, are the best available; but it must be noted that cinchona bark and quinine in various forms are included in the total. Several thousand hectograms of bark are said to be imported each year so that the figures given above are greater than the actual amount of the alkaloid quinine actually imported. The price of quinine sulphate has varied greatly and there are no records available in government or private files. The present wholesale price of quinine sulphate is 43 pesos (21.50 dollars United States currency) per kilogram (June, 1934). It is imported direct from Java.

[b] Estimated on the basis of value. Customs reports give only value and not amounts for these years.

[c] Importations of quinine not separately recorded for these years.

[d] Courtesy of the Bureau of Customs.

The estimate of 250 grains per case per year is lower than that of the League of Nations Health Organization,(3) which used 20 grams or about 308 grains per year as an average figure. But the league report estimates the quinine requirements of the Islands at only 1,305 kilograms as against the estimate of 32,400 kilograms quoted above. This discrepancy is due to the fact that in the league report the number of cases of malaria in the Philippines in 1930 is given as 64,251, an

impossible figure in view of the reported number of 15,144 malaria deaths in the same year. The disease malaria does not kill directly anything like 25 per cent of its victims. The deaths are probably not more than 1 or 2 in every 200 cases. Therefore, to account for 15,144 deaths there must have been from 1,500,000 to 3,000,000 cases, assuming the diagnosis of cause of death to have been accurate (which, of course, is often impossible). This question is discussed by one of us(2) elsewhere. We do not claim to know the correct figure, but we believe that at least 32,400 kilograms of quinine would be required to treat properly the cases of malaria now occurring each year in the Philippines. If this be true and if present quinine importations are continued, there remains a potential local market for at least 30,000 kilograms, more than 33 short tons, per year of locally produced totaquina.

It is a matter of common observation that very few cases of malaria in the provinces are adequately treated. The price of quinine sulphate varies but is commonly 1 centavo (0.5 cent United States currency) or more per grain as sold at retail, although it sells in bulk wholesale for 43 pesos (21.50 dollars) per kilogram. The retail price of one 5-grain tablet of quinine dihydrochloride is as high as 15 centavos in provincial *boticas*. At the usual retail prices a 250-grain treatment with quinine is out of the question for many thousands of malarious persons in the Philippines. Refined quinine is, in fact, an expensive luxury, a rich man's remedy. So too is atabrine, which retails for about 2.50 pesos per 15-tablet treatment. Totaquina might meet the need for a poor man's febrifuge, especially if it could be produced locally.

HISTORY

It would be interesting to recount at length the history of cinchona bark, but in this paper it must suffice merely to outline some of the important facts. This subject is admirably presented by Suppan.(4) According to this historian there is no solid foundation to support the interesting legend that we owe our first knowledge of the febrifuge qualities of cinchona to Peruvian Indians. For instance, from the discovery of Peru by Spaniards in 1513 to the beginning of the seventeenth century, none of the observers who wrote of their experiences in South America mentioned in any way a febrifuge bark. Moreover, the Indians themselves, after the Spaniards had learned of this bark, refused to use it as a medicine even after the properties were explained to them.

According to Suppan, it is highly probable that Jesuit missionaries were the first to discover the virtues of cinchona bark at some date and place still unrevealed. The first-known use of powdered cinchona bark to cure malaria was in 1630 when Don Juan Lopes de Cannizares, Spanish corregidor of Loxa, Peru, was successfully treated. In 1638, this corregidor sent some bark for the treatment of Countess Chinchon, wife of the viceroy of Peru. Having first tried it on a number of patients, her physician, Dr. Juan de Vega, gave some to the countess, with brilliant results. She was so grateful that she secured another supply and gave it gratis to all who applied at her palace. Hence its first popular name, *polvo de la Condesa.* So many applied for this remedy that she turned over to Jesuits the task of distributing it. The name then became *polvo de los Jesuitos* or *polvo de los padres.*

It is not clear exactly when the bark was first sent to Europe, but soon after 1640 it was widely known, largely because of the activity of Jesuit fathers, among whom Cardinal de Lugo was most enthusiastic. Hence another name—*pulvis cardinalis.* In 1649 this cardinal cured in Paris the dauphin who later became Louis XIV. This success naturally called widespread attention to powdered bark, which was becoming commonly known as *pulvis Peruanus* or *Peruvianum febrifugum.*

Just when the powdered bark first came to the Orient is uncertain, but it is recorded that in 1692 Jesuit priests in China used it to cure Emperor K'ang Hsi of a dangerous fever. It is not unlikely that supplies of the bark had also come to the Philippines at this early date. The Jesuits were absent from the Philippines from 1768 to 1852, but it seems very probable that they had imported supplies of this febrifuge prior to their departure. However, we have not been able to find any definite record of this. The fact that Filipinos have used bitter powdered bark from such local trees as *dita* [*Alstonia scholaris* (L.) R. Br.] as a febrifuge for more than one hundred years, is probably accounted for by a similarity in taste to powdered Peruvian bark.

Not until 1740 was a description of the fever-bark tree published in Europe. La Candomine, a French scholar, sent drawings and a description to Paris, and two years later Linnæus established the genus *Cinchona.* He misspelled the name of the countess whom he sought to honor, but the International Botanical Congress held in Paris in 1866 voted to retain his spelling.

In 1752 the Spanish Government began to organize the bark trade. It also searched for cinchona trees in regions other than Peru, finding them in Chile, Ecuador, and Bolivia. It may be noted that cinchona trees have never been found growing naturally in any part of the world other than this limited South American region.

The researches done by Humboldt and by Weddell were of great importance in spreading information regarding the collection and preparation of the barks and their botanical classification.

In 1820 two French pharmacists isolated quinine and cinchonine from the bark. The discovery of quinine, in particular, led to an enormous increase in the demand for bark. There followed a rapid and reckless destruction of cinchona trees, so that the supply was in danger of exhaustion. This suggested the possibility of cultivating cinchona elsewhere. Java was suggested in 1837 and India in 1839.

The Dutch were first to bring cinchona trees to the Orient. Hasskarl, superintendent of gardens in Java, left Peru in 1854, arriving in Batavia after a journey lasting four months. All but two of his plants died during the journey or afterwards. However, with his surviving specimens, augmented by later concessions, he was successful, and was knighted for his achievement.

The British had tried unsuccessfully in 1852 to raise cinchona from seeds sent to Calcutta; but in 1860, due to the efforts of Markham and Spruce, they were able to start growing cinchona in India. Their supplies came partly from South America and partly from Java. By 1866 there were more than 1,500,000 cinchona plants growing on the Nilgiri Hills, in southwest India, and this number had nearly doubled by 1872. In 1861 another cinchona plantation had been started in Ceylon.

The cultivation of cinchona in Java and India has continued, Java producing about nine-tenths of the bark of the world. The chief species now of commercial importance are *Cinchona ledgeriana* Moens, *C. calisaya* Weddell, *C. officinalis* Linnæus, *C. hybrida,* and *C. succirubra* Pavon. The first named has the highest quinine content.

About thirty alkaloids have been isolated from cinchona bark. The four chief alkaloids have been isolated as follows:

Quinine, 1820, isolated by Pelletier and Caventou.
Cinchonine, 1820, isolated by Pelletier and Caventou. (Gomez, 1810, made a partial separation of cinchonine.)

Quinidine, 1846, isolated by van Heijningen.
Cinchonidine, 1847, isolated by Winkler.

In both Ceylon and India the cultivation of cinchona was largely given up in order to produce the more-profitable tea, so that at present there is in reality a Dutch monopoly. Lately there has been a revival of interest in cinchona planting in India. Experimental cinchona is grown in certain areas of Africa, Indo-China, Malaya, and the Philippines, and in a few other regions. It does not appear that any unfair advantage has been taken of their monopoly by the Dutch. In fact there has been complaint about overproduction with insufficient returns on the investment. Certainly the Dutch have kept pace with the demand for quinine and have made every effort to reduce the costs of production.

Nevertheless, there is a serious world shortage in terms of the amount required by the estimated number of malaria patients in the world to-day. This is due in part to faulty distribution in malarious regions, but the chief reason is the fact that millions of sufferers cannot afford to buy quinine even at its relatively low price. Therefore, there is a very large field for the production of a still lower-priced product, such as totaquina. As noted above, there is a potential market in the Philippines alone for more than 30,000 kilograms a year. Totaquina cannot be considered to be a direct competitor of either quinine or the synthetic antimalaria drugs. It is required by malarious consumers who literally cannot afford to buy higher-priced drugs.

CINCHONA IN THE PHILIPPINES

The first reference we have found to cinchona growing in the Philippines is contained in a short note(22) about the "Jardin Botánico." The director of this garden in 1893 is reported to have said that he would like to acclimatize "Sinconas" at the foot of the Banahao mountains in Tayabas and also in Munang near Antipolo. There is nothing in this note to indicate that this was done, and we have found no further reference to it.

According to Fischer and Cenabre(5) it is safe to state that cinchona can be grown in the Philippines on areas properly located. They note that although it is commonly believed that cinchona is very delicate to handle, according to their ex-

perience and careful observation, the problem is not exceptionally difficult.

It appears that the first attempt to grow cinchona in the Islands was made in 1912 in Baguio, Mountain Province, and in Los Baños, Laguna, by the Bureau of Forestry. These attempts failed, as did several later ones. In 1916 three hundred plants were set out, but very few survived. In this same year Wester,(6) a horticulturist, stated, after visiting some cinchona plantations in Java, that the soil and climate of some parts of southeastern Bontoc appeared to be favorable for cinchona. He suggested that the Government consider the introduction of cinchona in those areas. It does not appear that his suggestion was followed, but an anonymous note(7) of 1919 stated that the introduction of cinchona plants into the Islands from India was being attempted by "the Igorot Exchange, a missionary institution in Sagada." Some 10 ounces of seed of *C. ledgeriana* was received from Ootacamund, Madras. According to the Right Reverend Bishop Mosher, this experiment apparently did not succeed, as no evidence of it remains in Sagada.

In 1927, according to Fischer and Cenabre(5) and Altamirano,(8) the Bureau of Forestry, with funds made available by the Reforestation Act, started a cinchona nursery in Impalutao, Bukidnon, Mindanao, using seeds from Java. This area has an altitude of about 762 meters, with an average rainfall of 112 inches annually, and temperature ranging from 17° to 29° C. The attempt has been successful, and an area of nearly 14 hectares has been planted to cinchona. There were 67,000 seedlings in the nursery ready for transplanting in 1933. There are now about 38,000 trees on the plantation itself, some 11,000 bearing fruit since 1930, and some 1,100 three or more years old. The species present are *C. ledgeriana, C. succirubra,* and *C. hybrida.*

Some bark from 5-year-old trees was analyzed with the following comparative results:(9)

TABLE 2.—*Cinchona bark analyses.*

Species.	Percentage of alkaloids.		
	Bukidnon.	India.	Java.
	Per cent.	*Per cent.*	*Per cent.*
Cinchona ledgeriana	9.6	8.52	8.6 (6.5–11.0)
Cinchona hybrida	4.74		
Cinchona succirubra	4.56	6.25	7.7

A second area in Bukidnon has been planted. This is at Barrio Alanib, in Malaybalay, and has an elevation of 1,067 to 1,372 meters. The trees are growing even better in this second location.

Quoting former Vice-Governor-General C. Butte,(9) who visited the plantations: "I can conceive of no production that would be more beneficial to all the people in these Islands, both from the commercial and the public standpoint, than the production of quinine within our country."

The authors of this paper would subscribe to this statement provided for "quinine" is read "totaquina." It is doubtful if the Philippines or any other country could successfully compete with the Dutch in the manufacture of the separated alkaloid quinine, but totaquina could certainly be produced and would find a very large potential market within the Islands.

TOTAQUINA

As a result of several conferences and considerable research the Malaria Commission of the League of Nations(10) suggested that a new cinchona product be prepared. This is to be called "totaquina," and is to be a standardized mixture of the combined alkaloids in cinchona bark, as follows:

Crystallizable alkaloids.—Not less than 70 per cent. Not less than 15 per cent of these alkaloids to be quinine.
Amorphous alkaloids.—Not to exceed 20 per cent.
Moisture.—Not more than 5 per cent.
Mineral matter.—Not more than 5 per cent.

Two types of totaquina have been prepared in commercial practice, as follows:

Type I.—Made by extracting and precipitating as an almost white powder the total alkaloids from the bark of *C. succirubra* or *C. robusta*, which can be cultivated in many malarious countries. This is the preferred type. It is the one we have in mind for the Philippines.

Type II.—Utilizing the residue remaining after quinine sulphate has been extracted from *C. ledgeriana* and bringing the preparation up to the required standard by adding sufficient quinine and other crystallizable alkaloids.

In this connection Howard,(11) who was in a position to know, stated that some of the advocates of cheap febrifuge mixtures appear to believe that there are vast quantities of cinchona products being wasted, which could be used (as in Type II totaquina). Howard said that he had no knowledge

of the existence of any hoards of residual alkaloids anywhere in the world.

The commission pointed out that the term "cinchona febrifuge," which was as heretofore applied to various mixtures of the alkaloids, should be discarded in favor of standardized totaquina. The name "quinetum" should be used only for a preparation containing quinine, cinchonidine, and cinchonine in equal parts.

The commission(10) reports that Giemsa(12) found totaquina a little more toxic than hydrochlorate of quinine when given in the same doses intravenously to rabbits. He found totaquina, both types, somewhat inferior to quinine in avian malaria (*Plasmodium relictum*). Giemsa, after a number of tests, concluded that the therapeutic efficacy of totaquina was directly proportional to the amount of quinine present.

Also cited by the commission are some tests by James(10, 13) in cases of benign tertian malaria intentionally induced by the bites of mosquitoes. The samples he used were composed as follows:

TABLE 3.—*Composition of samples used in tests by James.*

Constituent.	Type I.[a]	Type II.[b]
	Per cent.	*Per cent.*
Quinine	33.4	15.3
Cinchonine	21.7	55.4
Cinchonidine	34.7	5.7
Quinidine	0.0	5.2
Total crystallizable alkaloids	89.8	81.6
Amorphous alkaloids	5.3	13.4
Moisture	0.76	1.9
Ash	0.6	2.1

[a] Type I consisted of total alkaloids of *C. succirubra*.
[b] Type II was composed of quinine residues.

James's(10) results may be quoted as follows: (*a*) One dose of 5 grains (0.3 gram) of totaquina of either type has practically no effect on the fever or parasites. It is necessary, therefore, to use a single dose of 10 grains (0.6 gram) for the test. (*b*) A single dose of 10 grains of totaquina Type I produces the same effect in aborting the fever and in reducing the parasites as that produced by a single dose of 5 grains of quinine. (*c*) A single dose of 10 grains of totaquina Type II has practically no effect in aborting the fever or in reducing the parasites. With this type of totaquina it is necessary to use a single

dose of 20 grains (1.2 grams) to produce the same effect as that produced by a single dose of 5 grains of quinine.

James(10) concludes as follows:

> It appears from these results that totaquina Type I (total alkaloids of *C. succirubra* or *C. robusta*) when used in ordinary clinical therapeutic doses for curative purposes—e. g. 1.2 gram (20 grains) daily for five to seven days—should give about the same good result as is given by quinine in the same doses. If this is so, and if this type of totaquina can be obtained more cheaply than quinine, it would be advantageous to use it for general purposes instead of the single alkaloid.

The method by which Type I in the above tests was prepared is given by Groothoff and Henry(13) as follows:

> The process used was that described by Schwyzer, Fabrikation der Alkaloide, Berlin (1927), which depends on the extraction of the alkaloids from the ground bark, previously mixed intimately with slaked lime, by boiling benzene. From the benzene solution the alkaloids are in time removed by agitation with dilute sulphuric acid. The aqueous alkaloidal solution so obtained is decolourized by heating with charcoal, filtered, and, while still hot, run into excess of sodium hydroxide solution to precipitate the alkaloids. After standing for several hours the precipitate is collected, washed with water and allowed to dry in the air.

The samples obtained were nearly white or pale cream-colored powders, completely soluble in dilute sulphuric acid, giving a fluorescent solution.

The British Pharmacopoeia(14) includes totaquina (totaquine), giving its characters, tests for identity and purity, and its assay.

According to Dawson,(15) who cites Henry,(16) the four common cinchona alkaloids are really two pairs of optical isomers, quinine and quinidine forming one pair, and cinchonidine and cinchonine the other. Quinine and cinchonidine are levorotatory, and quinidine and cinchonine are, respectively, their dextrorotatory isomers. Dawson,(15) after reviewing the literature, states that quinine and quinidine are of practically equal value in the therapy of malaria of any species. All four alkaloids are about equal in their efficacy against chronic benign tertian malaria.

Totaquina would replace such preparations as quinetum, first extracted in Java in 1874, which was not standardized and did not prove to be economical. It was a coarse preparation of mixed alkaloids. So, too, a cinchona febrifuge similar to but better than quinetum was never well standardized. The Indian cinchona febrifuge came to be a residual alkaloidal rather than

a total alkaloidal preparation. Nevertheless, cinchona febrifuge has had considerable use and value. It has become the most important of the commercial alkaloid preparations.

It may be noted that the usefulness of the other alkaloids of cinchona in malaria therapy has been well known since 1866–1867 when three Madras Commissions in India reported favorably after extensive experiments. There has been ample confirmation by Fletcher(17) and others. Field(18) has recently reported some observations that led him to conclude that totaquina gives excellent results in malaria therapy. The subject is also reviewed by Sinton,(19) especially as to the place of cinchona febrifuge in malaria therapy.

PREPARATION AND ANALYSIS OF TOTAQUINA

The totaquina used in this investigation was prepared from the ground bark of *Cinchona ledgeriana*, which was grown at the Philippine Bureau of Forestry Station at Impalutao, Bukidnon, Mindanao. As previously stated, the bark of this *Cinchona* species has a higher percentage of total alkaloids than those of *C. succirubra* and *C. hybrida*, which are also grown there.

The results of our analysis of Philippine *Cinchona ledgeriana* bark, according to the method of Howard and Chick,(20) are given in Table 4. As shown by the data, this bark has a rather high percentage of total alkaloids, more than half of which is quinine.

TABLE 4.—*Analysis of the dried bark of Philippine Cinchona ledgeriana.*

Constituent.	Per cent.
Quinine	5.51
Cinchonidine	1.45
Quinidine	1.94
Cinchonine	Trace.
Amorphous alkaloids	0.72
Total alkaloids	9.62

Preliminary experiments were carried out in order to ascertain a satisfactory method for making totaquina from cinchona bark. As a result of our experiments, we made three preparations which are recorded as A, B, and C.

The method used for making totaquina A was essentially the same as that recommended for the preparation of "quinetum" from *Cinchona succirubra*.(21) The result was not so satis-

factory, as we obtained a dark brown powder that was only partly soluble in dilute sulphuric acid.

Our preparation of totaquina B gave better results. The method for this preparation was very similar to that employed in making totaquina A, except that the precipitated alkaloids were dissolved in dilute sulphuric acid (about 0.2 *N*) and filtered to remove insoluble impurities. The acid filtrate was heated and then decolorized with purified charcoal. The hot solution was then made strongly alkaline with sodium hydroxide solution in order to precipitate the alkaloids. When cooled, the precipitate was collected on a filter, washed with water, and dried in a vacuum oven below 50° C. Totaquina B has a light brown color but is not completely soluble in dilute sulphuric acid, though much more nearly so than totaquina A.

Our third preparation, totaquina C, gave the best results. This product was made in the following manner: The finely powdered cinchona bark was macerated with dilute hydrochloric acid. The mixture was then poured into a percolator and the extraction completed with water as the solvent. The acid percolate was treated with a sufficient amount of sodium hydroxide solution to precipitate the coloring matter as flakes. After the coloring matter had been removed, the solution, which was still strongly acid, was made strongly alkaline with sodium hydroxide solution. The mixture was set aside overnight and the alkaloids that separated from the turbid liquid were removed by filtering. They were washed with water until the washings were almost colorless, and dried. This represented the first portion of the totaquina. The filtrate from the first portion contained a considerable quantity of alkaloids in suspension. These were precipitated (salted out) with sodium chloride. After standing for several hours, the supernatant liquid was removed and the precipitate collected on a double filter and washed with water. The precipitate was treated with dilute sulphuric acid and filtered to remove impurities. The acid filtrate was decolorized with animal charcoal and the hot solution precipitated with an excess of sodium hydroxide. The precipitate was removed, washed with water, and dried, after which it was extracted with hot ethyl alcohol (95 per cent). The alcoholic extract was distilled to remove the excess alcohol. The residue was evaporated to dryness and incorporated with the first fraction of the alkaloids previously obtained. A kilogram of powdered cinchona bark gave 90 grams of totaquina C or a yield of

9 per cent. A complete assay of the bark (Table 4) gave 9.62 per cent of total alkaloids. The yield of totaquina C is, therefore, about 93 per cent of the total alkaloids in the bark.

This product, totaquina C, was a pale brown powder, which dissolved easily and completely in dilute sulphuric acid, giving a blue fluorescent solution.

The alkaloidal constituents of totaquina B and C were determined according to the method of Howard and Chick.(20) The results are recorded in Table 5. Instead of determining the quinine by weighing the dried quinine sulphate, we dissolved the salt in hot neutral ethyl alcohol and titrated the solution with alcoholic potassium hydroxide (0.1 *N*), using phenolphthalein as indicator.

TABLE 5.—*Analysis of totaquina preparations from the bark of Philippine Cinchona ledgeriana.*

Constituent.	Totaquina preparations.	
	B	C
	Per cent.	*Per cent.*
Quinine	42.01	53.90
Cinchonidine	18.82	21.98
Quinidine	10.08	14.71
Cinchonine		
Total crystallizable alkaloids	70.91	90.59
Amorphous alkaloids	15.54	5.85
Total alkaloids	86.45	96.44
Moisture	5.14	1.55
Ash	3.23	1.91
Organic impurities (by difference)	5.18	0.10

As shown by the data (Table 5) totaquina C is a better product than totaquina B because it contains not only more of the total crystallizable alkaloids but also more quinine.

The cost of preparing totaquina C in our laboratory was approximately 16.07 pesos per kilogram. This includes the average cost of cinchona bark (87 centavos per kilogram), as given by the Bureau of Forestry (see below), and incidental expenses for chemicals and labor. The estimated expenses are itemized as follows:

	Pesos.
Cinchona bark, 11.1 kilos	9.66
Concentrated hydrochloric acid, 610 cc	1.60
Sodium hydroxide, 597 g	1.36

	Pesos.
Sodium chloride, 1 kg	0.20
Concentrated sulphuric acid, 100 cc	0.20
Charcoal, 500 g	0.65
Ethyl alcohol, 1 kilo	0.20
Filter paper, 10 sheets	0.20
Total cost of materials	14.07
Labor	2.00
Total expenses	16.07

This is a laboratory cost. In commercial production the cost would doubtless be less. As stated above the wholesale price of quinine sulphate in bulk is 43 pesos per kilo (the Government pays 40 pesos). In 5-grain tablets the wholesale price is 14 pesos per 1,000 tablets (the Government pays 12 to 13 pesos). This price for tablets is practically equivalent to the price for the powder.

Boticas, or drug stores, in the provinces, charge 1 centavo a grain for quinine sulphate, as an average minimum. The price is not infrequently doubled in places where the demand is greatest. At 1 centavo a grain the retail cost of quinine sulphate becomes 154 pesos per kilogram.

Obviously, totaquina can be sold for much less than that. We submit the following estimate of the retail cost of a kilogram of totaquina as a fair approximation:

	Pesos.
Cinchona bark (This allows a fair profit to the grower. See table below.)	9.66
Preparing the totaquina (This allows a 15 per cent profit on production costs of 6.41 pesos as set forth above.)	7.37
Wholesale price per kilo	17.03
Allowance for retailer's profit and expenses, 20 per cent	3.40
Retail price of totaquina to consumer, per kilo	20.43

This estimated retail price of 20.43 pesos per kilogram of totaquina is higher than it would be if production were carried on commercially, but it compares very favorably with the present retail price of 154 pesos for quinine sulphate. If retailers were now limited to a 20 per cent profit, the retail price of quinine sulphate would be 51.60 pesos per kilogram, more than two and a half times the liberally estimated totaquina price. But since established prices are not easily changed it is perhaps fair to

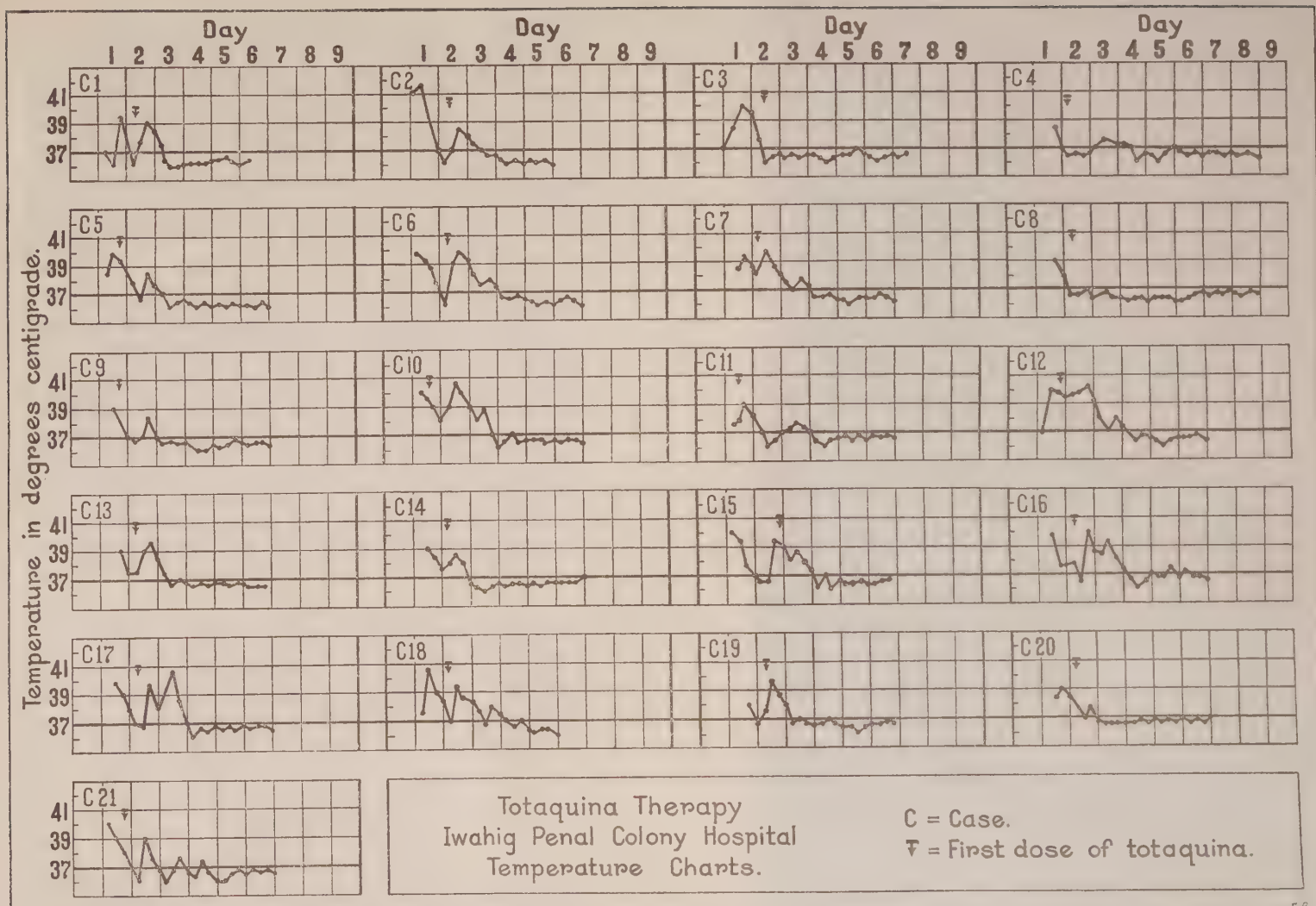

FIG. 1. Temperature charts, totaquina therapy, Iwahig Penal Colony Hospital. Patients were hospitalized for at least fifteen days. The part of the temperature charts not shown above presented only normal temperature.

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contrast the present actual retail price of quinine of 154 pesos with the estimated price of 20.43 pesos for totaquina, which as a new drug being introduced by the Government could from the first be subject to some price restrictions under official supervision. If this comparison is used the totaquina could be sold to the people for something like one-seventh of the present price of quinine! In this case a 250-grain treatment would cost not 2.50 pesos, as at present, but only about 35 centavos, a price within the means of the malarious poor. If sold to the people by the Government, eliminating the retailer's profit, the price would be even less.

CLINICAL TEST OF TOTAQUINA

In this study of totaquina, twenty-one frank cases of malaria having positive blood examination for parasites were selected for trial. Particular attention was given to its effect upon chills and fever and upon schizonts and gametocytes of the two common types of malaria infections; namely, the benign tertian, caused by *Plasmodium vivax*, and the malignant tertian, caused by *Plasmodium falciparum*. Observations were made as to any untoward effects which could be attributed to the drug. Careful clinical histories as well as physical examinations of the cases for trial were noted, and blood examinations made at intervals before and during the administration of the drug. Thick and thin smears were stained with Giemsa, and each thin smear was examined for at least thirty minutes if negative.

DOSAGE

Totaquina was given in the same dosage as quinine in the treatment of malaria; namely, 0.6 gram three times a day for adults. It is not so bitter as quinine and hence can be administered to children in papers with lactose. Children less than 2 years of age were given 0.2 gram three times a day. Totaquina sample A was used to treat cases 1 and 2; sample B, cases 3 to 16, 19, and 20; sample C, cases 17, 18, and 21.

MALARIA CASES GIVEN TOTAQUINA IN IWAHIG PENAL COLONY HOSPITAL

CASES OF BENIGN TERTIAN TYPE

Cases 1 to 18 were all diagnosed as benign tertian (case 13 was a mixed infection with subtertian). All were Filipino males unless otherwise stated. Fever charts are given in fig. 1, and a record of blood smear examinations in Tables 6 and 7, and

fig. 2. The physical examination was normal unless otherwise stated.

CASE 1. C. L., AGE 53 YEARS, ADMITTED APRIL 11

Clinical history.—Fever and chills accompanied by headache and profuse sweating regularly every afternoon for three days. Spleen three-fingers' breadth, palpable at left costal margin on normal respiration.

Treatment.—Totaquina, 0.6 gram three times a day for fifteen days.

Observation.—Two days after administration of the drug temperature became normal. Spleen receded in size until at the time of discharge it was palpable on deep inspiration.

CASE 2. F. P., AGE 33 YEARS, ADMITTED APRIL 11

Clinical history.—Chills and fever every other day for three days. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for fifteen days.

Observation.—One day after administration of the drug temperature became normal. No more chills. The 3d day no malarial parasites could be seen in thick smear.

CASE 3. R. S., AGE 24 YEARS, ADMITTED JUNE 13

Clinical history.—Fever and headache for two days. Spleen palpable on deep inspiration.

Treatment.—Totaquina, 0.6 gram three times a day for ten days, then reduced to 0.3 gram. t. i. d. until the 15th day.

Observation.—After administration of the drug there was no further rise of temperature. Two days after, the blood became negative for malarial parasites and remained so. The 12th day spleen no longer palpable.

CASE 4. C. C., AGE 54 YEARS, ADMITTED JUNE 14

Clinical history.—Fever, chills, and headache every afternoon for four days. Spleen not palpable.

TABLE 6.—*Blood-smear examinations. Totaquina therapy. Iwahig Penal Colony Hospital.*[a]

[+, Positive, but fewer than 1 per 100 leucocytes.]

Case.	Initial.	Date.	Parasites per 100 leucocytes.		Species.
			Asexual.	Gameto-cytes.	
1	C. L.	Apr. 11	68	0	*Plasmodium vivax.*
		Apr. 12	4	0	Do.
2	F. P.	Apr. 11	238	0	Do.
		Apr. 12	20	0	
3	R. S.	June 13	+	0	Do.
		June 14	+	0	
		June 15	0	+	
4	C. C.	June 15	11	0	Do.
		June 16	1	0	
		June 17	1	0	

[a] A complete record of negative examination is given in text fig. 2.

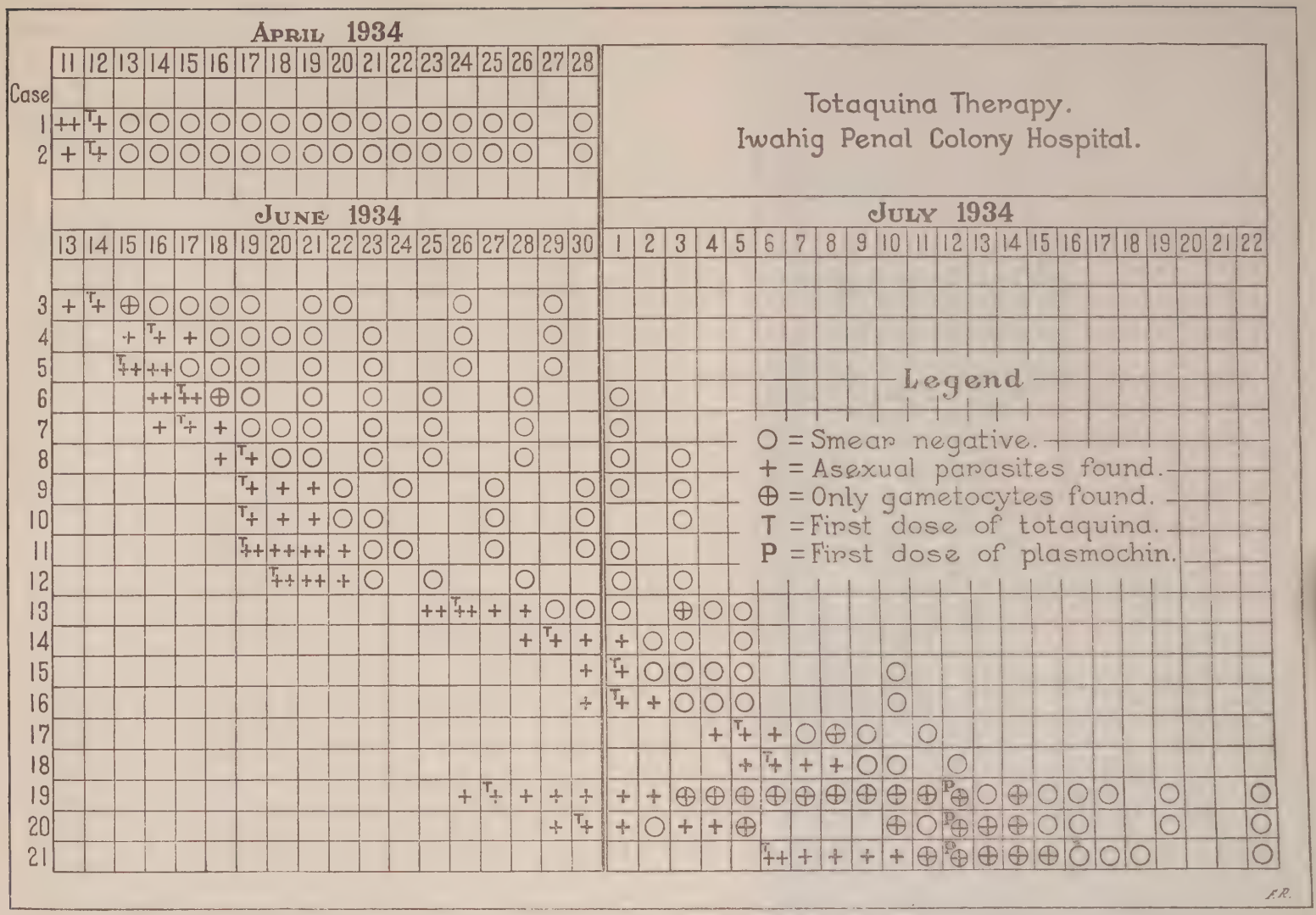

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FIG. 2. Totaquina therapy, Iwahig Penal Colony Hospital. Blood-smear chart.

TABLE 6.—*Blood-smear examinations. Totaquina therapy. Iwahig Penal Colony Hospital.*[a]—Continued

Case.	Initial.	Date.	Parasites per 100 leucocytes.		Species.
			Asexual.	Gametocytes.	
5	O. P.	June 15	48	0	*Plasmodium vivax.*
		June 16	138	2	
6	R. R.	June 16	306	0	Do.
		June 17	68	0	
		June 18	0	+	
7	F. C.	June 16	250	0	Do.
		June 17	208	2	
		June 18	20	0	
8	A. P.	June 18	5	0	Do.
		June 19	4	0	
9	F. G.	June 19	147	0	Do.
		June 20	86	0	
		June 21	4	0	
10	B. O.	June 19	170	0	Do.
		June 20	6	0	
		June 21	6	0	
11	F. A.	June 19	80	0	Do.
		June 20	4	0	
		June 21	+	0	
		June 22	+	0	
12	A.	June 20	18	0	Do.
		June 21	4	0	
		June 22	+	+	
13	P. C.	June 25	316	10	Mixed *Plasmodium vivax* and *falciparum.*
		June 26	256	42	
		June 27	4	2	
		June 28	2	+	
		July 3	0	+	
14	S. M.	June 28	302	0	*Plasmodium vivax.*
		June 29	68	0	
		June 30	1	0	
		July 1	+	0	
15	B. d. V.	June 30	57	0	Do.
		July 1	2	0	
16	E.	June 30	209	0	Do.
		July 1	40	0	
		July 2	4	0	
17	S. D.	July 4	130	0	Do.
		July 5	2	0	
		July 6	10	4	
		July 8	0	+	
18	S. T.	July 5	205	0	Do.
		July 6	104	0	
		July 7	40	0	
		July 8	+	0	

[a] A complete record of negative examinations is given in text fig. 2.

TABLE 6.—*Blood-smear examinations. Totaquina therapy. Iwahig Penal Colony Hospital.*[a]—Continued

Case.	Initial.	Date.	Parasites per 100 leucocytes.		Species.
			Asexual.	Gameto-cytes.	
		June 26	148	0	*Plasmodium vivax* and *falciparum*.
		June 27	74	0	
		June 28	4	2	
		June 29	+	+	
		June 30	2	2	
		July 1	2	2	
		July 2	2	2	
		July 3	0	+	
		July 4	0	+	
19	S. M.	July 5	0	+	
		July 6	0	+	
		July 7	0	+	
		July 8	0	+	
		July 9	0	+	
		July 10	0	2	
		July 11	0	+	
		July 12	0	+	
		July 13	0	0	
		July 14	0	+	
		June 29	90	0	Do.
		June 30	4	0	
		July 1	+	0	
		July 2	0	0	
		July 3	+	0	
20	J. B.	July 4	+	0	
		July 5	0	+	
		July 10	0	2	
		July 11	0	0	
		July 12	0	+	
		July 13	0	+	
		July 14	0	+	
		July 6	86	0	Do.
		July 7	4	2	
		July 8	2	2	
		July 9	6	6	
21	M. C.	July 10	2	2	
		July 11	0	4	
		July 12	0	4	
		July 13	0	2	
		July 14	0	3	
		July 15	0	4	

[a] A complete record of negative examinations is given in text fig. 2.

Treatment.—Totaquina, 0.6 gram three times a day for eight days, then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Two days after administration of the drug temperature became normal. The 4th day blood smear became negative.

CASE 5. O. P., IGOROT, AGE 22 YEARS, ADMITTED JUNE 15

Clinical history.—Fever, headache, and chills with profuse perspiration each afternoon for two days. Spleen palpable on deep inspiration.

Treatment.—Totaquina, 0.6 gram three times a day for nine days, then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Two days after administration of the drug temperature became normal and blood negative. After ten days' treatment spleen receded and could no longer be palpated on deep inspiration.

CASE 6. R. R., AGE 33 YEARS, ADMITTED JUNE 16

Clinical history.—Fever and headache, vomiting for two days. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for seven days, then reduced to 0.3 gram until the 15th day.

Observation.—Two days after administration of the drug fever subsided and blood became negative the next day.

TABLE 7.—*Disappearance of parasites following totaquina therapy.*[a]

Parasites disappeared—	Cases.	
	Asexual parasites.	Gametocytes.
	Cases.	*Cases.*
In 1 day	6	0
In 2 days	5	4
In 3 days	6	2
In 4 days	1	1
In 5 days	2	0
In 6 days	1	0
In 8 days	0	1
In 10 days	0	1
In 18 days	0	1
Total	21	10

[a] In the history records the day is usually counted from that of admission. In the above table it is counted from the time the first dose of totaquina was given.

CASE 7. F. C., AGE 31 YEARS, ADMITTED JUNE 16

Clinical history.—Fever and chills followed by profuse perspiration every afternoon for three days. Spleen palpable on deep inspiration.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Two days after administration of the drug temperature became normal and blood smear negative the following day. Spleen still palpable on deep inspiration, at time of discharge.

CASE 8. A. P., AGE 27 YEARS, ADMITTED JUNE 18

Clinical history.—Headache, fever, and chilly sensation for two days. Spleen palpable on deep inspiration.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—The drug was administered at the time of subsidence of fever, which did not rise again. The next day blood smear became negative. The 6th day spleen no longer palpable on deep inspiration.

CASE 9. F. G., AGE 30 YEARS, ADMITTED JUNE 19

Clinical history.—Fever, headache, and chills in the afternoon for two days. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for seven days, and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Two days after administration of the drug temperature became normal. Blood negative the 4th day.

CASE 10. B. O., AGE 27 YEARS, ADMITTED JUNE 19

Clinical history.—Fever, headache, and chills coming every afternoon regularly for three days. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—After three days' administration of the drug temperature went to normal, and blood smear became negative.

CASE 11. F. A., AGE 47 YEARS, ADMITTED JUNE 19

Clinical history.—Fever, headache, and chills for two days each morning. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Three days after administration of the drug temperature became normal. Blood became negative the 5th day.

CASE 12. A., IGOROT, AGE 24 YEARS, ADMITTED JUNE 20

Clinical history.—Fever, headache, and chills, followed by profuse sweating coming every other day. Spleen palpable on inspiration.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Three days after administration of the drug temperature became normal and blood smear negative. The 9th day, spleen could no longer be palpated on inspiration.

CASE 13. P. C., AGE 37 YEARS, ADMITTED JUNE 25

Clinical history.—Fever, headache, and chills coming each afternoon for two days. Patient had chills in the ward. Spleen three fingers' breadth below left costal margin.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Mixed infection, tertian and subtertian. Two days after administration of the drug temperature became normal. The 5th day blood smear became negative. Crescent seen the 9th day, blood thereafter negative. After six days of treatment spleen became palpable on inspiration.

CASE 14. S. M., AGE 27 YEARS, ADMITTED JUNE 28

Clinical history.—Fever, headache, and chills for four days regularly every afternoon. Spleen two fingers' breadth below left costal margin.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until 15th day.

Observation.—One day after administration of the drug temperature became normal. The 5th day blood smear became negative. The 13th day spleen could no longer be palpated on inspiration.

CASE 15. B. V., AGE 21 YEARS, ADMITTED JUNE 30

Clinical history.—High fever, headache, and vomiting for two days. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—One day after administration of the drug blood smear became negative and temperature normal.

CASE 16. E., IGOROT, AGE 40 YEARS, ADMITTED JUNE 30

Clinical history.—Fever, headache, and chills every afternoon for three days. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Two days after administration of the drug temperature became normal and blood smear negative the 4th day.

CASE 17. S. D., AGE 24 YEARS, ADMITTED JULY 4

Clinical history.—Fever, headache, and chills for two days coming every afternoon. Spleen tender and palpable on deep inspiration.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Two days after administration of the drug temperature became normal and blood smear negative the 5th day. Spleen not palpable on day of discharge.

CASE 18. S. T., AGE 34 YEARS, ADMITTED JULY 5

Clinical history.—Fever, headache, and chills every afternoon for three days. Spleen palpable on deep inspiration.

Treatment.—Totaquina, 0.6 gram three times a day for seven days and then reduced to 0.3 gram t. i. d. until the 15th day.

Observation.—Three days after administration of the drug temperature became normal and blood smear negative the 5th day.

CASES OF MALIGNANT TYPE

Cases 19 to 21 were all diagnosed as subtertian malaria. Fever charts and results of blood-smear examinations are given in text fig. 1 and Table 1. Physical examination normal unless otherwise stated.

CASE 19. S. M., AGE 60 YEARS, ADMITTED JUNE 26

Clinical history.—Fever, headache, and chills, followed by profuse perspiration every afternoon for three days. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day for nineteen days, then reduced to 0.3 gram t. i. d. until discharged two days later.

Observation.—Two days after administration of the drug temperature became normal. Young asexual forms disappeared the 8th day, but gametocytes persisted until the 16th day of treatment, when plasmochin (1 tablet

t. i. d.) was added to totaquina. Gametocytes then disappeared in forty-eight hours.

CASE 20. J. B., AGE 25 YEARS, ADMITTED JUNE 29

Clinical history.—Fever, headache, and chilly sensation for six days every afternoon. Spleen not palpable.

Treatment.—Totaquina, 0.6 gram three times a day. After thirteen days' treatment there were still crescent forms in the peripheral blood. Plasmochin simplex then added to treatment, one tablet three times a day.

Observation.—One day after administration of the drug temperature became normal and young asexual forms disappeared the 6th day. Crescent forms appeared in increasing numbers. Two days after adding plasmochin to totaquina the blood smear became negative.

CASE 21. M. C., AGE 1 YEAR 5 MONTHS, ADMITTED JULY 6

Clinical history.—Fever occurring irregularly for about two weeks. Spleen extends to umbilicus.

Treatment.—Totaquina, 0.2 gram combined with lactose in papers administered with milk three times a day for fourteen days. The 7th day plasmochin was added, one tablet once a day for six days.

Observation.—Three days after administration of the drug, temperature became normal and asexual forms disappeared the 6th day. Crescents began to appear in increasing numbers. When plasmochin was added to treatment, gametocytes began to decrease in number and after four tablets were given, gametocytes were no longer seen.

COMMENTS

Totaquina in doses of 0.6 gram three times a day usually relieves the patient of symptoms within two days, at most three days. The temperature returns to normal and no more chills occur. The schizonts and gametocytes of benign tertian disappear within the same period, schizonts being cleared first.

The effect of totaquina on the crescent form of *Plasmodium falciparum* is negligible, just as in quinine or atabrine medication, but it is definite on schizonts of the malignant type. As was observed, addition of plasmochin to the treatment clears the blood of crescents. There were no untoward effects of totaquina observed, such as ringing in the ears, vertigo, vomiting, or deafness, frequently observed in intense quinine medication.

We observed that with administration of the drug there was a tendency toward increase in the number of gametocytes in subtertian cases. This has been observed also with quinine and atabrine. It appears that the response of parasites to the drug leads to increased formation of gametocytes.

Whether totaquina would give a thorough cure so as to prevent relapses, we are not yet in a position to say, as the period of ob-

servation after patients were discharged from the hospital was too short and the chances of reinfection were too many.

The fact that totaquina is much less bitter than quinine (although more bitter than euquinine) makes it easily administered to children.

SUMMARY

This paper briefly reviews the history of cinchona and its alkaloids, especially quinine. It points out that cinchona cultivation has been found practicable in the Philippines by the Bureau of Forestry. It calls attention to the recommendation of a committee of the Health Organization, League of Nations, that a standard extract of total alkaloids of cinchona be developed as a cheap substitute for the purified quinine. The name "totaquina" was recommended for this new product.

This paper reports the easy and inexpensive preparation of totaquina at the Bureau of Science from Philippine cinchona bark. It further reports successful clinical trials with totaquina at the Iwahig Penal Colony Hospital.

Finally, this paper suggests that the preparation and distribution of Philippine totaquina could be developed into a local industry of importance, and that it might have a notable effect in reducing the incidence of malaria.

CONCLUSIONS

1. It is not improbable that if every case of malaria occurring in one year in the Philippines could be given a 250-grain treatment there would be needed at least 30,000 kilograms more specific febrifuge than is now imported.

2. A 250-grain treatment with quinine sulphate in the provinces costs from 2.50 to 5 pesos (1.25 to 2.50 dollars United States currency). The greater the need, the higher the price. Quinine dihydrochloride retails for from two to four times as much as the sulphate. These retail prices are far more than the average farmer in the provinces can pay. Quinine and the synthetic drugs plasmochin and atabrine may therefore be called a rich man's remedies. There is no probability that much more quinine can be paid for than is now imported.

3. The Bureau of Forestry has demonstrated that cinchona will grow in the Philippines and will give as good a yield of alkaloids as that grown elsewhere.

4. From our studies we conclude that the standardized total-alkaloid-extract of cinchona, recommended by the Health Organ-

ization of the League of Nations and called "totaquina," can be prepared locally from Philippine cinchona easily and inexpensively.

5. We conclude from some clinical tests that this Philippine totaquina is probably about equal to quinine sulphate in its therapeutic value against malaria. We are not able to form any conclusion as regards relapses, but we found that totaquina subdues the acute attack effectively and without untoward effects. It does not destroy the crescents of subtertian malaria, being similar to quinine and atabrine in this respect.

6. We conclude that, allowing a fair profit to the grower of cinchona, the manufacturer of totaquina, and to the retailer, this Philippine totaquina could be sold to the people at not more than 35 centavos (0.175 dollar) per 250-grain treatment.

7. Contrasting 35 centavos with the present retail price of from 2.50 to 5 pesos for a 250-grain treatment with quinine sulphate or atabrine, we conclude that the local production of totaquina would materially aid in combating malaria in the Philippines.

8. We also conclude that the growing of cinchona and the manufacture of totaquina might have considerable economic importance to the Islands, being capable of becoming sizeable new industries.

DATA FURNISHED BY THE PHILIPPINE BUREAU OF FORESTRY

	Pesos.
Cost of 5,016 seedlings to be planted in 1 hectare with spacing of 1.5 by 1.5 meters at 11 pesos per 1,000	55.18
Average cost of clearing, preparing, and planting 1 hectare	114.00
Total	169.18
Interest due 169.18 pesos for seven years at 7 per cent, compounded annually	102.52
Annual expenses for up-keep and supervision, excluding land rent and taxes at 127 pesos per hectare, plus the interest on the amount at 7 per cent, compounded annually	1,099.46
Cost of harvesting and curing the bark of 2,721 trees at 0.10 centavos per tree	272.10
Total expenses	1,643.26
Yield of 2,721 trees at 0.96 kg per tree, 2,612 kg at 0.87 centavos per kg	2,272.53
Profit at the end of the seventh year	629.32
Income per hectare per year	89.90

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ILLUSTRATIONS

TEXT FIGURES

FIG. 1. Temperature charts, totaquina therapy, Iwahig Penal Colony Hospital. Patients were hospitalized for at least fifteen days. The part of the temperature charts not shown above presented only normal temperatures.

2. Totaquina therapy, Iwahig Penal Colony Hospital. Blood-smear chart.

THE TECHNIC OF HANDLING MOSQUITOES [1]

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EIGHT PLATES AND TWELVE TEXT FIGURES

INTRODUCTION

The authors have recently prepared a series of papers to facilitate the practical identification of Philippine *Anopheles*.(1, 2, 3) This discussion of technic is added to supplement those papers, in the hope that it will increase their usefulness. It is intended to be simple enough for ordinary field use in the Islands and yet sufficiently comprehensive to assist those who may desire to do more than merely identify a given larva or adult.

We have found the following references of particular value with regard to the technic of handling mosquitoes: Boyd,(4) Christophers,(5) and Gater.(6) Many other references could be given. Most general books on tropical medicine include a discussion of this subject.

We cannot resist stating at the very outset that, to be successful, an inspector or other officer interested in malaria requires not so much finesse in technic as curiosity, enthusiasm, and determination. Field work in malariology will not permit white collars or polished shoes. It is frequently arduous, dirty, and damp.

Furthermore, there are almost as many technical methods of dealing with mosquitoes as there are malariologists. We can only present those which have seemed best to us. They should

[1] This paper has been prepared as a part of the program of Malaria Investigations, a project which has been jointly supported by the Bureau of Science, Manila, and the International Health Division of the Rockefeller Foundation. The Bureau of Health has coöperated through the assignment of the junior author as chief field director.

be modified and improved to suit individual needs and not followed slavishly.

TECHNIC OF HANDLING OVA

Individual anopheline or aëdine mosquito eggs are so small that they are rarely found in natural breeding places. *Culex* mosquitoes lay their ova in rafts (text fig. 1), and these are easily seen floating on the surface of stagnant water; but *Anopheles* ova are usually deposited singly and escape detection (text fig. 2).

Ova for study may be obtained from gravid females. When first laid they are white, but they soon become black. They vary in length from 0.6 to 0.8 millimeter, and each species has definite morphological characters. For some countries keys to the *Anopheles* ova have been prepared. For example, in Europe six varieties of *A. maculipennis* have been identified by their

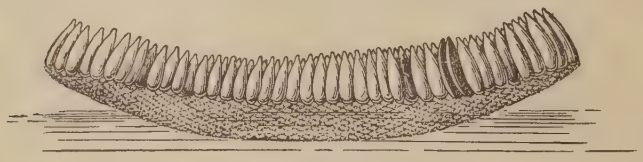

FIG. 1. *Culex* egg raft.

eggs.(7) It would be well to study the ova of Philippine *Anopheles*, a subject on which nothing has been published. Differentiation is made on the basis of size, shape, and position of the lateral floats, as well as the design on the dorsal surface of the ovum. Color may also be important. (See text fig. 2.)

According to Hackett(7) anophelines with ripe eggs will readily lay them in small cotton-stoppered vials, 50 by 20 millimeters, on wet filter paper or pressed cotton. "The eggs are usually laid in a small heap and they can be dispersed and at the same time preserved by dropping 2 per cent formalin on them from a pipette or dropping bottle (five or six drops). The eggs can be kept on wet cotton in small entomologist's tin specimen boxes, 1¼ in. in diameter, which have a glass set in the lid, through which the eggs can be examined. Washers or rubber rings cut from tubing the same diameter as the tin box serve to keep the cotton at the bottom from falling against the lid if the box should be overturned or sent by post." We have not tried this method.

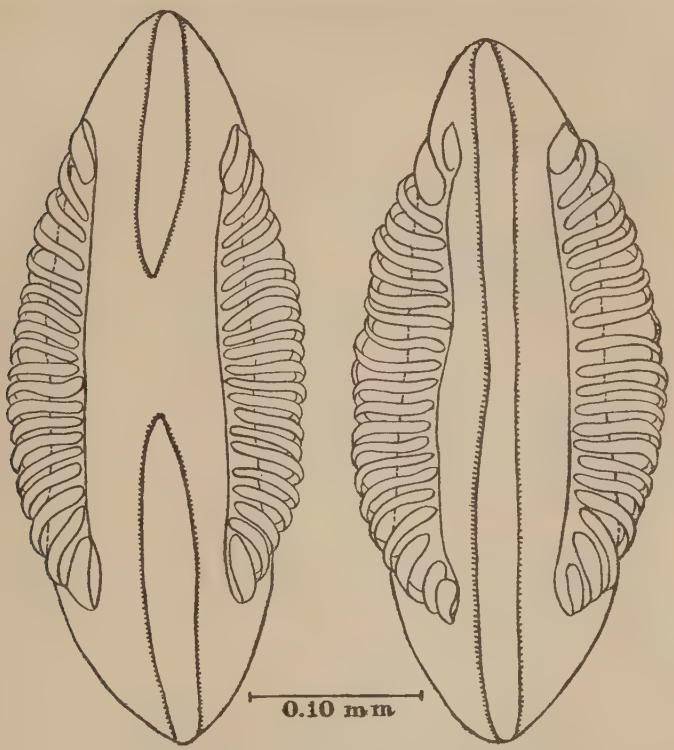

FIG. 2. Two types of eggs of *Anopheles minimus* var. *flavirostris*.

LARVÆ

COLLECTING LARVÆ (PLATE 1, FIGS. 1 AND 2)

The following equipment is essential for collecting mosquito larvæ:

1. Dipper.—Any sort of a receptacle may be used, from a coconut shell to a standardized copper dipper specially made for mosquito surveys. It is best as a rule to use a tin or enamel dipper having a long hollow handle into which a cane may be thrust. In some places a shallow pie tin or deeper cake tin may be useful. In other places, such as shallow water, a large spoon is most serviceable. If comparative quantitative studies are being made, a standardized dipper of about 400 cubic centimeters capacity should be used.

We have found very useful an Italian type of tin dipper having a screen window protected by an outer shell (text fig. 3). This screen permits water to be poured out of the dipper without loss of larvæ and thus facilitates the collection. If the inside of the dipper is painted white it is easier to see the larvæ.

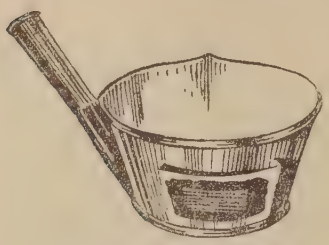

FIG. 3. Special type of dipper.

2. Pipette or spoon for removing larvæ from the dipper.—An ordinary medicine dropper with the narrow end cut off is satisfactory, although wider special collecting pipettes may be purchased and are more desirable. Narrow medicine droppers are not suitable for removing large culicine larvæ or pupæ, in fact these may be killed when drawn into the ordinary pipette. In such cases, if no large pipette is available it is better to use a spoon.

3. Collection bottle.—Some sort of a collection bottle is needed in which to carry the larvæ from the field to the laboratory. Any wide-mouthed bottle that can be stoppered will do. We have found half-pint mason jars satisfactory. These can be fastened to the belt.

4. Carrying case.—A carrying case is required for the bottles. We have used a simple wooden case with a rack to support six bottles upright (Plate 1, fig. 3). An army bag is useful in the mountains or difficult terrain. It can be slung over the shoulders or strapped to the back and permits the hands to remain unencumbered.

5. Notebook.—It is essential to keep an adequate record of the collections. A notebook and a pencil should be carried in the field so that notes can be made at the breeding place.

6. Bolo.—It is always wise to carry a bolo or other large knife to cut undergrowth and to kill snakes.

There are some practical points about larva collecting which should be noted. In the first place the type of breeding place to be searched is of importance. If the object of collecting is to make a complete anopheline and culicine survey, no accumulation of water can safely be overlooked. Even tree holes and miscellaneous rain-catching hollows and receptacles must be examined. *Aëdes mosquitoes* will breed in a gill of water, even within houses, as in ant guards and flower pots.

If only anophelines are wanted, the following types of breeding places may be included in the survey:

Running water in creeks, streams, rivers, ditches, canals, and springs.

Stagnant fresh water in wells, lakes, ponds, pools, ditches, and swamps, as well as in tree holes, rock pools, temporary rain puddles and hoof prints, and in miscellaneous barrels and tubs. Rice fields are a prolific source of *Anopheles* mosquitoes.

Salt water in salt ponds, fishponds, lagoons, swamps, and river mouths.

Finally, if the object is a malaria survey, collections may be limited to running water, including springs and spring-fed wells.

The proven malaria-carriers in the Philippines are *A. minimus* var. *flavirostris* and *A. maculatus*. The former is of paramount importance, while the latter has rarely been incriminated. Both species breed in running water, and neither has been taken in either salt water or completely stagnant water, except in fresh residual overflow pools in stream beds.

Anopheles minimus var. *flavirostris* is most abundant in foothill streams, close to partially shaded edges, especially where there are bamboo roots. It has not been found above 2,000 feet altitude. *Anopheles maculatus* has been taken up to 5,000 feet altitude. It is most abundant along the edges of partially shaded forest streams.

In collecting it is best to approach the actual breeding place without disturbing it, for the larvæ are easily alarmed and quickly submerge or scatter. They can remain submerged for several minutes. Therefore, while wading in the stream, the collector should try not to send waves toward the breeding place. If feasible, he should stand on the bank while dipping. The dipper should not hover over a breeding place but make decisive, clean-cut dips. It is to be remembered that the anopheline larvæ are on the surface, and surface water should therefore get into the dipper as expeditiously as possible. If dipping is done where there are roots, the dipper should be so manipulated that surface water will rush into it from among the roots, carrying with it the larvæ. It may be necessary to dip several times in one spot before the larvæ are taken.

In contrast to this "sucking" maneuver in which the water is drawn out from among roots, one may in some places use a sweeping movement of the dipper, skimming a considerable area

of the surface of the breeding place in one arclike sweep. When there is débris or a floating log, the dipper may be quietly submerged beside it so that currents of water will carry the larvæ into the dipper, somewhat as in the case of larvæ from roots, described above. If the dipper overflows, many larvæ may be lost. If the water is turbid or full of débris, it may take several minutes to remove the larvæ from the dipper, as they are prone to remain for a time at the bottom. It is more difficult to take pupæ than larvæ, the former being quicker to see danger and to escape from it.

Small puddles and hoof prints may be stirred first, before dipping. The muddy water will furnish a background against which the larvæ, after a little time, are easily seen and removed directly with a pipette or spoon. For tree holes a long pipette is useful. One may easily be made from glass tubing and a large rubber bulb. If the tree hole is dry some of the material in the bottom should be taken. The ova of tree-hole mosquitoes resist drying, and sometimes, when the removed material is put in water in the laboratory, larvæ will appear.

For wells the dipper used by Russell and Santiago(8) is very useful. It has a long handle with a hinge and set screw near the dipper so that it may be given various angles to adapt it to existing circumstances.

The capture of only a few first-stage larvæ does not seriously incriminate a breeding place for it does not prove that larva will grow to maturity in the given site. But if large larvæ or pupæ are taken, or if larval skins are seen, then it may be assumed that the breeding place is of importance for the species taken.

If ten adequate dips along a meter of stream bank are fruitless, that site may safely be called negative at the time of examination.

Negative results may be due to inexperience in collecting, to recent disturbances, such as flushing, abrupt change in water level, agitation of the breeding place, application of larvicide, or to other causes. Therefore, a place found negative should not be omitted on subsequent visits, but subjected to repeated scrutiny.

Field notes should include not only the date and the general type of breeding place, such as stream, pool, or spring, but also a statement about shade—whether dense, medium, light, or absent. It is well also to record whether or not the water is clear or muddy, what type of bottom, what type of vegetation

is along the bank, what water plants are present, and whether natural enemies, such as top minnows or dragon-fly larvæ, are seen.

If quantitative studies are being made, the larva of different stages should be counted separately and the number of dips recorded. In all cases the place of collection should be carefully stated; that is, name of area, kilometer post, or distance and direction from a fixed, well-known point. If a detailed survey is being made it is essential to prepare a sketch map of the area, including roads, settlements, and bodies of water.

Finally, collecting larvæ successfully requires considerable practice. Many a place has been called negative because the would-be collector was not adept. The dry-foot man is never to be trusted because it is utterly impossible to make adequate collections without frequently entering the water. Experienced collectors sometimes rely on the appearance of a place and dip only in the likely places. This is dangerous, as one can be easily fooled. Larvæ are sometimes found in abnormal sites, especially in a dry season.

TRANSPORTING LARVÆ

Anopheline larvæ can be transported considerable distances, if reasonable care is exercised. We have found that the half-pint mason jars mentioned above are suitable. Several may be moved about in a wooden (not metal) collecting box which has a rack inside to keep the bottles upright, and a handle to facilitate carrying. If the distance to be covered is not great and the road is fairly smooth, the bottles may be two-thirds full of water with perhaps a small amount of green algæ added to minimize the motion of the water. If the larva must be transported over longer distances, it is well not to have the bottles more than a quarter full, and it is essential to add algæ. The bottles must be correctly labeled, and no natural enemies should be included in them. If jars are not available, bamboo joints may be used satisfactorily. They are especially useful for larvæ from rock holes and artificial containers. We have transported living larvæ in such bamboo joints from Mindanao to Manila, a four days' journey. A good number of *leucosphyrus* and near-*leucosphyrus* emerged from this collection. The top of the container was covered with gauze held in place by rubber bands.

Sometimes living larvæ may be sent through the mails in a special type of bottle, stoppered, but having a glass tube piercing the cork and extending about halfway into the bottle. The

quantity of water used is such that, no matter what the position of the bottle is, the surface of the water does not touch the open end of the tube (text fig. 4).

Boyd(4) states that for transportation over very rough ground or on horseback it is a good plan to transfer the larvæ to a bottle

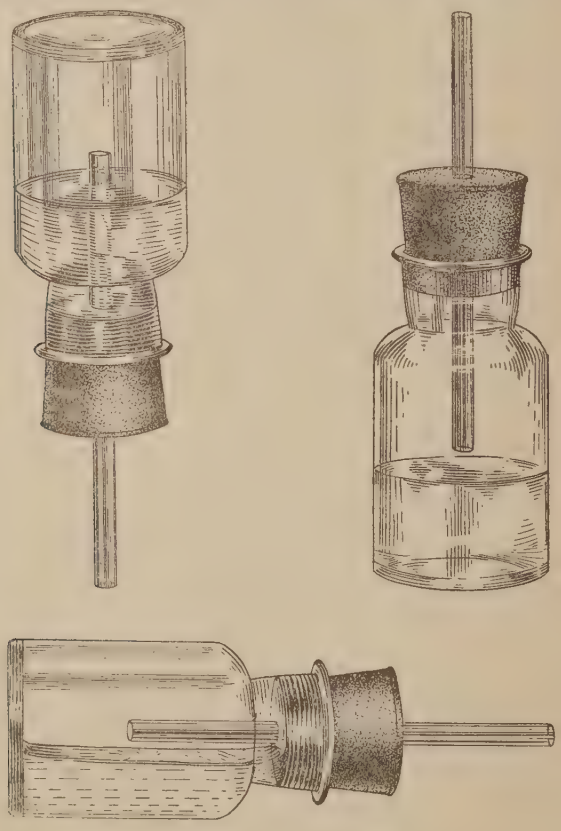

FIG. 4. Larva shipping bottle.

which has been freshly filled with water and emptied, so that the inside is wet. The larvæ become deposited nearly immobilized in the film of moisture and the surplus water is discarded. The bottle is then tightly sealed. A second bottle is filled with water from the breeding place, and this furnishes a supply to be used in the laboratory for rearing the larvæ.

REARING LARVÆ (PLATE 2, FIG. 2)

When the larvæ have been taken to the laboratory they should be transferred to properly numbered or labeled bowls or pans, which have more surface area than the bottles. Special attention must be paid to light, temperature, and food. For proper development the larvæ require some direct sunlight, but in the Tropics it is sufficient to expose them only from about 6 to 7 a. m. For the rest of the day it is better not to have them directly in the sun. An appreciable rise in the temperature of the water above normal will result in high mortality.

Numerous foods have been recommended for larvæ. We have found a mixture of Löffler's dehydrated blood serum and litmus milk satisfactory for all species of mosquito larvæ. Very small amounts are used. Another good food is yeast. A piece about half a centimeter square may be put in the bowl on a support of some kind so that the yeast is held about a centimeter or so below the surface of the water.

Where these special foods are not available good results may be had with natural algæ, finely chopped cockroaches, or hay infusion. The last is made simply by adding some hay or rice straw to water and allowing it to soak. Algæ from salt-water ponds, boiled and allowed to stand for a few days, form a white scum which is an excellent food for larvæ. It is somewhat odorous and should be protected by gauze from flies. Regardless of what food is used the water must not be allowed to get foul and should be changed as necessary.

It has seemed to us that aërating the water accelerates development of the larvæ. This is done simply by means of a pipette, repeatedly but gently injecting air below the surface of the water in the bowl.

It is sometimes a good plan to separate the various stages of larvæ, for the older may attack and devour the younger ones. Culicine larvæ should be kept separately as many species are cannibalistic. As a general rule, unless the species is a rare one, it is best to discard the small larvæ and to rear only those of the third and fourth stage. The smaller ones require much attention and give unsatisfactory results.

For rearing large numbers of *A. minimus* var. *flavirostris* larvæ Russell and Santiago (9) erected a wire cage in a stream at the bank. The water of the stream flowed through a very fine screen at each end of the cage, simulating natural conditions.

In the laboratory a flow of water through a basin may be secured from a receptacle used as a reservoir above the basin and connected with it by a thick thread of lampwick. This wick or thread extends from the water in the reservoir down to and through the basin containing the larvæ, and hangs over the edge of this basin. Water flows by capillary attraction and gravity down the thread to the basin and from there to waste.

It is necessary to inspect the bowls of larvæ each day. Pupæ are transferred to individual vials or test tubes about one-third full of water, and lightly plugged at the top with cotton. Pupæ do not feed, but are very susceptible to high temperatures and to rough handling. A small twig may be put in the vial or test tube as a resting place for the adult when it emerges. The vials or test tubes must be properly numbered or labeled.

Adults should be transferred to cages, lantern chimneys, or dry, clean vials, also properly labeled. Leaving them in the vial with water usually results in destruction of fringe spots and scales of the wings. But this transfer should not be made for about six hours, so that the adult will have time to dry out.

It is frequently a good plan to save the last skin, or pelt, which is molted when the larva becomes a pupa. This pelt is preserved, as noted below, and affords a very useful check on the identification when its identification is compared with that of the adult which emerges from the pupa. Making a diagnosis from both the larval skin and the adult avoids any possibility of confusion.

Each collector will by experience find out the best way to rear his larvæ under the conditions prevailing in his own laboratory. Surprisingly good results can be had with makeshift equipment, provided the collector has sufficient interest in the subject.

PRESERVING LARVÆ OR PUPÆ

It is always necessary to keep in mind the fact that the identification of larvæ or pupæ depends for the most part on minute body hairs. These are fragile and are easily rubbed off by rough handling. Therefore, the specimens must be handled with delicacy. It is a good rule to manipulate them singly and not to attempt to save time by carrying several in one manipulation of a pipette.

For temporary preservation the larvæ or pupæ may be killed in hot, but not boiling water (temperature about 65° C.). They may be transferred from the collecting bottle to the hot water and removed as soon as dead. Leaving them longer than neces-

sary in the hot water tends to bend the bodies and to make further examination or manipulation difficult. They may then be picked up by a pipette and put into a 10 per cent solution of acetic acid where they will retain their characters for a few days, at least. If chloral hydrate is available a more suitable temporary preservative is made by dissolving 57 grams of chloral hydrate in water and adding 6 cubic centimeters of glacial acetic acid and enough water to make 100 cubic centimeters of the preserving fluid.

Either of these temporary preserving fluids is suitable for forwarding specimens from the provinces to Manila, but neither is advisable for permanent preservation.

If permanent preservation of unmounted larvæ or pupæ is desired, MacGregor's solution is suitable. This is made by dissolving 5 grams of borax in water, and adding 2.5 cubic centimeters of glycerin, 100 cubic centimeters of a 40 per cent solution of formaldehyde, and enough distilled water to make 1 liter. After thorough mixing the solution is filtered. Larvæ or pupæ, after being killed in hot water, are placed in this solution, in small vials or bottles tightly corked and sealed with wax or paraffin. It is always wise to put a pencil-written label inside the vial and to add another label, written in India ink, on the outside of the vial. If this outside label is brushed with Bureau of Science book varnish it will be impervious to weathering and to cockroaches. We have found MacGregor's solution very satisfactory. It keeps the larvæ flexible, whereas in 10 per cent formalin they tend to become bent and somewhat brittle.

If considerable numbers of larvæ are to be preserved in one vial it is better to make a double transfer from water to a small beaker of the preservative and thence to a vial of fresh preservative. This prevents undue dilution. Larvæ and pupæ so mounted will keep their characters for many years, even under tropical conditions.

Larval pelts may be preserved temporarily in 70 per cent alcohol in individual vials, properly numbered. We prefer the borax-formalin solution described above. For permanent preservation it is best to mount the pelt, as described below.

MOUNTING LARVÆ OR PUPÆ

Larvæ and pupæ may be mounted for temporary or permanent use. Temporary mounts may be made of either living or preserved specimens. Living larvæ or pupæ may be mounted in a drop of water on a glass slide for diagnosis. If they are

handled with reasonable care they may be returned to the bowls and reared into adults. If it is necessary to use a cover slip on a living larva it is better to put a small piece of paper or broken cover glass, or a hair, under the slip to avoid injury to the specimen. The careful use of ice water or of weak chloroform water will tend to immobilize the larva without killing it.

Preserved larvæ may be mounted temporarily for examination in a drop of the preserving solution. It is usually better to use a cover slip and to mount the larvæ dorsum uppermost near the middle of the slide.

Permanent mounts give more satisfaction than the temporary ones. They may be made from either living or preserved larvæ or pupæ. We prefer Gater's(6) method of mounting. Based on his description the process for living larvæ is as follows:

1. Prepare the mounting fluid. This is done by placing 8 grams of picked gum arabic in 10 cubic centimeters of distilled water in a beaker which is covered and kept in a warm, but not hot, place, such as an incubator, stirring it occasionally until it is dissolved. After it has dissolved add 74 grams of chloral hydrate, stir and replace in incubator until dissolved. Then add 5 cubic centimeters of glucose syrup (98 grams of bacteriological glucose dissolved in 100 cubic centimeters distilled water) and 3 cubic centimeters glacial acetic acid. Stir and allow to stand. Then filter through No. 5 Whatman paper in a Buchner funnel, using a suction pump. If this is impossible, allow the solution to stand until all sediment has settled and then decant the upper portion. [If this solution cannot be made in a local laboratory a small supply may be obtained from the entomologist at the Bureau of Science or from the malaria control section of the Bureau of Health, Manila.]

2. Transfer the larva from the collecting bottle or rearing bowl to the center of a glass slide. We have found it a good plan to trace the outline of a standard slide on a piece of cardboard and on this trace the outline of a cover glass, marking the center. This furnishes a guide for mounting. The specimen should be placed with the head toward the one doing the mounting. A little practice will soon result in perfectly aligned slides so that there will be complete uniformity of appearance in a box of slide mounts.

3. Remove as much water as possible, first with a capillary pipette and then with a piece of blotting paper until the specimen is almost dry. Avoid touching the larva as much as possible. If it gets out of position it can be maneuvered into place by tapping and tilting the slide. Experienced technicians, however, can arrange and rearrange the larva and its hairs using very fine dissecting pins and a binocular to get the best results. Allow the larva to adhere to the glass so that it will remain in place during subsequent manipulations.

4. With a fine glass rod place a drop of the mounting medium, described in paragraph 1 above, over the larva as it lies with dorsal part of head and body uppermost. When the larva is dead add another drop

of mounting fluid and carefully place a clean cover glass over the specimen. The fluid decreases in volume as it dries so an excess should be used. This medium sets hard in about a week but the larva will be cleared and suitable for examination in a few hours.

Slightly better results may be had if the larva is killed in hot water and then kept overnight in the chloral hydrate medium described under temporary preservation above.

5. Add the proper label at the right end of the slide as the head of the specimen is towards the one doing the mounting. Brush the label with a collodion or book varnish to preserve it.

6. When the mounting medium is dry the cover glass should be ringed as neatly as possible with a cellulose varnish such as Duco. This is best done with a turntable but may be accomplished satisfactorily by hand.

Mounting larvæ which have been preserved is done in the same way as just described, except that, if MacGregor's solution is the preservative, it is advisable on removing the larva to place it first in water for an hour and then overnight in the chloral hydrate temporary solution described above. Larvæ which have been temporarily preserved in acetic acid may be directly mounted but are better if given a preliminary bath in this chloral hydrate solution.

The arrangement of the specimen on the slide so that the hairs are properly disposed requires a careful technic, which comes only after considerable practice. It is essential to use just the right amount of mounting fluid as larger or smaller drops will spoil the mounting. We have found satisfactory three drops from an ordinary toothpick when the smaller end of the pick is dipped for about one-third of its length into the fluid, and the initial drop is allowed to fall into the container as the toothpick is removed. Care should be taken that the fluid spreads evenly over the specimen which should be entirely submerged. The cover slip is dropped squarely on the top of the fluid.

The use of forceps in placing the cover glass may not be satisfactory as the glass may easily slip out of position. Thumb and forefinger are usually better tools.

Larval pelts may be mounted temporarily in a drop of the 70 per cent alcohol preservative. Permanent mounts of these pelts are extremely difficult and require considerable practice. Gater(6) gives detailed instructions which we are not including here as we suggest that, if permanent mounts are desired, the pelts be forwarded in the preserving fluid either to the entomologist of the Bureau of Science or to the malaria control section of the Bureau of Health, Manila.

IDENTIFYING LARVÆ AND PUPÆ

For the identification of the larvæ of Philippine *Anopheles* we have recently prepared a wall chart, distributed by the Bureau of Health, and an illustrated key,(2) which should be consulted. Other papers of importance in this regard are those by Manalang,(10) Baisas,(11) and King.(12) The pupal characters of Philippine species are being studied by the junior author.

Before attempting to identify a larva it is, of course, necessary to be acquainted with the larval characters used in a key. We have described this subject at length in our key.(2)

Having become familiar with the larval characters, one should proceed with the identification systematically. Head, thorax, and abdomen should be examined in order and the characteristics of the important hairs, the tergal plates, and the pecten noted. We prefer to use the low power of a compound microscope in the identification of larvæ. With a little practice it becomes possible to recognize all of the essential characters. One of us(13) has used a microscope mounted on a tripod by Darling. This was taken into the field and the species of larvæ in a given breeding place were identified at once.

When the characters have been studied it becomes possible, by means of the key, to make an identification; but usually more than one character is required for positive diagnosis. There are many individual variations. Furthermore, although Barber et al.,(14) Holt and Russell,(15) and Russell(16) have published fairly comprehensive reconnaissance studies, there has not been an adequate survey of Philippine *Anopheles*. Therefore, the possibility of encountering a new species or variety is always present. If a careful study of a specimen fails to establish a clear diagnosis, it should be sent to the malaria control section of the Bureau of Health.

DISSECTING LARVÆ

It is sometimes desirable to dissect larvæ to determine their diet or to observe whether or not they have ingested a larvicide mixture, such as Paris green and charcoal.

Apparently, most anopheline larvæ ingest a heterogeneous diet, consisting of any sort of inert or living material small enough for passage through their gullets; but their nutrition seems to depend on the digestion of such green algæ as *Euglena* and *Cosmarium*, diatoms, rotifers, ciliates, and other protozoa. No studies have been reported on the diet of Philippine *Anopheles* larvæ, although it is a matter of importance.

The dissection of a larva offers little difficulty. With dissecting needles the anterior part of the thorax is cut off. Then the abdomen is nicked just anterior to the last segment and the gut attached to this terminal segment drawn out. The gut is transparent, and if it is flattened in a drop of water or saline solution under a cover slip, such contents as Paris green, charcoal, or protozoa can readily be distinguished.

As noted by Boyd and Foot(17) the digestion of a larva proceeds very rapidly, and owing to the disturbance of the water in a collecting bottle the larva may not feed during transit. For this reason the gut may be nearly empty by the time the larva is examined in a laboratory. Therefore, the specimen for examination of gut contents should at the time of capture be put into a solution of 10 per cent formalin 9 parts, and glycerin 1 part. It is better to use only fourth-stage larvæ. Then, in the laboratory, after making an identification of species, the gut may be removed and crushed in a little water on a glass slide. Several specimens from larvæ of the same species may be put together and a microscopical analysis of the total gut contents made.

In larval food studies it is usual also to collect about a half pint of water from the breeding place, and, after shaking and centrifuging in the laboratory, to examine the sediment for plankton.

IMAGINES, OR ADULTS

COLLECTING ADULT MOSQUITOES

The following equipment is essential for collecting adult mosquitoes (Plate 3, figs. 1 to 3).

Flashlight.—Adult mosquitoes usually are caught either at night or in dark places in the daytime. Therefore, a flashlight is indispensable. An ordinary two- or three-cell dry-battery light is suitable. If the light can be focused it may be slightly more convenient, but this is not essential. A supply of batteries and bulbs should be available. Incidentally, as noted below, a powerful flashlight is very useful in the laboratory for the examination of adults.

Catching tube.—Many types of catching tubes are used. The simplest and the one which has given us good results is a small vial measuring about 8 by 1.5 centimeters. Test tubes may be used. With a little skill such a vial or tube can be placed over a resting anopheline and quickly corked with the disturbed insect inside. Only one insect should be kept in a vial.

Another very useful collecting tube is easily made by attaching a length of rubber tubing over one end of a glass tube. The tube should have a diameter of about 8 to 10 millimeters and a length of about 30 centimeters. The rubber tubing is about 45 centimeters long. The end of the glass tube over which the rubber is tied should be first covered by a piece of gauze. Suction is made by the mouth at the end of the rubber tubing as the free end of the glass is placed near a mosquito, which is easily sucked into the tube and then gently blown into a collecting vial or killing bottle. Large numbers of insects can be caught by this tube in a very short time. Even flying mosquitoes can sometimes be caught. This method is somewhat rougher than hand-catching with individual vials, but, with practice, it is satisfactory.

Various types of special catching tubes are sold by entomological supply houses. One which has had widespread use is a thick-walled, hollow, glass cylinder about 15 centimeters long and 3 centimeters in diameter. It has one inverted conical end which is perforated by a small hole. The other end is covered with a piece of gauze and corked. Through a hole in the cork there is a short glass tube on the outer end of which is a rubber suction bulb. Mosquitoes are sucked into this tube and cannot easily escape.

Carrying box or case to transport the insects to the laboratory.—Any suitable box or bag or even the collector's pockets may be used to carry the collecting vials or tubes of mosquitoes. It must be remembered that exposure to direct sunlight quickly kills mosquitoes, as does prolonged drying or jarring.

Notebook.—It is essential to label the catch properly and to keep a field record of the collecting places. If quantitative studies are being made, the length of time spent in searching should be recorded.

It is very unusual to catch *Anopheles* adults in, on, or under Filipino nipa houses in the daytime. These insects enter such houses freely at night and when blooded have been observed to linger inside until daybreak, but when it becomes light the *Anopheles* mosquitoes seek other daytime shelter. Nipa houses are light, dry, and airy, whereas the insect prefers to rest where it is dark, damp, and quiet. Typical daytime resting places for local anophelines are small undercut caves along stream banks, well-shaded mossy stone walls such as those near old churches or cemeteries, small sheltered cuts, or the interstices of the inner

wall of a shallow well, on the moist walls of partially covered cisterns, under cement structures, and sometimes inside strong-material houses in a dark, damp room. Adult anophelines may be caught by day or by night and will be discussed under these two headings.

DAYTIME CATCHES

Philippine *Anopheles* have a flight range of at least 2 kilometers. They are not active by day but only at night, and their daylight hours are spent quietly in such shelters as described above. Therefore, if daytime catches are to be made, typical shelters must be searched. This presents no difficulties, except that occasionally snakes will be found in the same small caves chosen by the mosquitoes. An efficient way to collect anophelines by day is to walk down a stream known to be a breeding place, methodically searching each undercut with a flashlight. Not only the stream-breeding species will be found, but also others from nearby rice fields and pools. If there are well-shaded old stone walls in the vicinity they may also be a fruitful source of anophelines.

Artificial daytime shelters can be made by lining boxes with moist earth. The earth-lined box traps that we have used measured about 1 by 0.5 by 0.5 meter.(21) The bottom and one end of the box are removed so that the floor of the trap is earth. With wire screening to hold it, a layer of moist earth about 1 inch thick is applied to the inside of the remaining three sides and the top. The open end is partly closed with a black cloth, which hangs to within 5 or 10 centimeters of the ground. This provides the insect with a dark, damp, quiet, and earthy resting place, such as it naturally seeks. Such traps may be placed near breeding places, under houses, or wherever it is desired to trap anophelines. The insects are easily taken with any of the usual collecting tubes, as they rest quietly on the inside.

NIGHTTIME CATCHES

Anophelines in the Philippines may be caught at night on the walls of bedrooms, on mosquito nets, or feeding on carabaos or cows. Many local species, including the malaria-carrying *A. minimus* var. *flavirostris*, will feed on carabaos, and can be easily caught while doing so. By the use of a fairly tame beast and a flashlight good catches can be made with any of the usual collecting tubes from about 7.30 p. m. until just before daylight.

We have had success with animal-baited traps. These are screened structures of various sizes, usually made somewhat larger than is necessary to accommodate one carabao. The carabao is tethered inside, surrounded by a bamboo railing to prevent damage to the screening. The one or two doors of the trap are left open but are closed just before daybreak. Many thousand anopheline mosquitoes have been taken daily inside such traps. It is wise to have an overhanging roof to protect the walls of the trap from rain, and to darken it somewhat so that the insects will not be too eager to escape at daybreak.

Smaller traps, baited with a man sleeping inside but under a mosquito net, have also given good results. In one case we used a screened room in a nipa house, closing the windows at daybreak.

The malaria-carrying *A. minimus* var. *flavirostris* apparently will take cow or carabao blood about as readily as it will take human blood, so that animal-baited traps are suitable even for this species.

Baited traps located in the center of a control zone will serve as useful indicators to measure the success or failure of control projects. If the malaria-carrying mosquito is taken in the trap in considerable numbers the control project is not succeeding.

If cattle were kept in sheds in the Philippines, such cattle sheds would doubtless be suitable places for catching adults, but we have rarely seen any sort of cattle shed in use locally. The usual shelters, if they exist at all, consist merely of a roof supported by posts, with no side walls. Strong-material houses attractive as daytime-resting places for mosquitoes are also the exception rather than the rule in the provinces.

Sometimes jungle species of *Anopheles* can be taken by sitting quietly in the dark in a suitable place, collecting those which attempt to feed. This is a dangerous procedure in malarious regions.

We have not had much success using collecting nets. Apparently the local species do not rest to any extent among grasses or shrubbery in the daytime. Occasionally it is possible to net considerable numbers of anophelines as they swarm beside a breeding place at dusk.

We have not been successful with any of the usual plain box traps reported in the literature. Only our earth-lined box trap mentioned above has given us good results.

It requires a little experience to detect adult mosquitoes as they rest in their natural sheltering places. One must look closely and carefully, but it is not long before practice makes it easy not only to see the insects but even to differentiate *Anopheles* from *Culex* and sometimes to identify immediately certain species.

TRANSPORTING ADULT MOSQUITOES

Whatever collecting tube is used it is necessary to have a box or a bag for transporting the insects to the laboratory. They must be kept out of the direct sun and should be handled as gently as possible. Various types of field transporting boxes have been used, and one may be easily devised to meet the needs. Wood is better than tin for a field box as it will not conduct heat so well.

We have had considerable success shipping living mosquitoes by mail in individual glass vials, packed carefully in a mailing container. The vials we have found best measure 8 by 1.5 centimeters. A hole is punched in the cork and a moist cotton plug is put in the hole. A strip of blotting paper is wedged inside along the length of the vial and a mat of the same substance is placed at the bottom. We have had a high percentage of success by this method, up to three days in transit by train and bus, with outside shade temperatures up to 37° C. (98.2° F.). As a matter of convenience the mosquitoes were caught and shipped in the same vials. After receipt in the laboratory the mosquitoes, still in their individual vials, if put in an ice box, will in many cases remain alive for a week (text fig. 5).

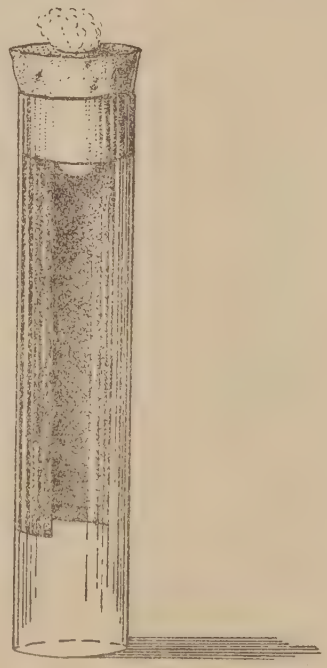

FIG. 5. Vial for shipping living adults.

We have had some success shipping live mosquitoes in a box patterned after Barraud's, but we have come to the conclu-

sion, after comparative tests, that the individual vial method is a better one.

Incidentally, if the mosquitoes being transported are from malarious regions, very great care must be exercised in opening the container which may contain deadly mosquitoes. It has been our practice to use a small screened cage having a cloth-sleeve entrance, so that both hands could be put inside the cage and any manipulation procedures carried on without danger of the mosquito escaping into the room. Of course, in many cases it will suffice to kill the insect in its vial by applying chloroform to the cotton plug in the cork, as soon as it is received in the laboratory.

The chief advantage in shipping mosquitoes alive is that they may be dissected in a central laboratory. It also facilitates studies of the ova.

If it is desired to ship dead mosquitoes they may easily be killed with chloroform vapor. Sufficient exposure should be allowed so that the insects are actually killed and not merely stupefied. They are then put in pill boxes, one collection to a box, unless there are so many that undue crowding would result. Ordinary cardboard pill boxes may be used. A still better box is the small 0.25- or 0.5-ounce tin container such as is used to gather samples of fæces for diagnosis, or to hold various ointments.

A little naphthalene, wrapped in a layer of cotton, is put in the bottom of the container. Lens paper is better. The insects are placed on top of the lens paper, or cotton, and are covered by another layer of the same material, and the tin or pill box is capped with its cover. Cotton expands and tends to put undue pressure on the specimens, so that if used it should be sparingly.

A minute drop of creosote may be used instead of naphthalene as a deterrent against ants and other insects, which quickly destroy unprotected specimens in the Tropics.

REARING ADULT ANOPHELINES (PLATE 2, FIG. 2; TEXT FIGS. 6 AND 7)

We have discussed the care of larvæ in the laboratory up to the emergence of the adult insect. The adults that have emerged in the pupal vials may be transferred to lantern chimneys, which serve well as cages. The smaller end is covered with gauze held in place by adhesive tape. The lower, larger end rests in a Petri dish, the bottom of which is covered with filter paper. Raisins, boiled with sugar, seem to make acceptable

food for the insects. One boiled raisin put on top of the gauze will remain succulent for a considerable time. Not more than 25 to 30 mosquitoes should be kept in one cage at a time. It

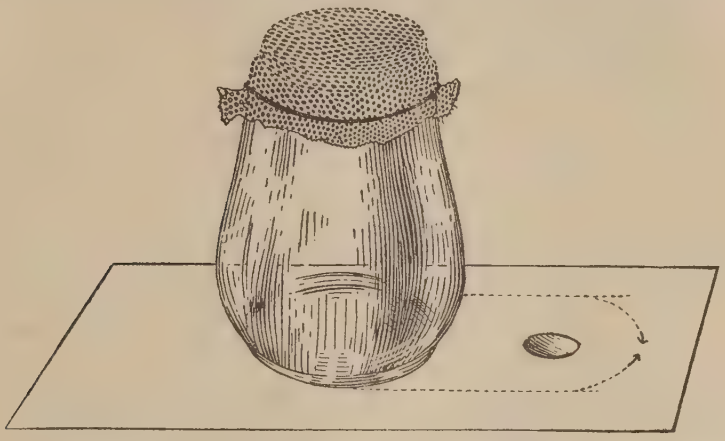

FIG. 6. Lantern-chimney cage with manipulating tin. Dotted lines show position of base of chimney when adults are being inserted or withdrawn.

is important to protect the cages from ants which can make serious inroads during a single night, destroying and carrying off the adults.

It is not necessary to use a lantern chimney. A small screened cage of any type can be used, provided food and water are

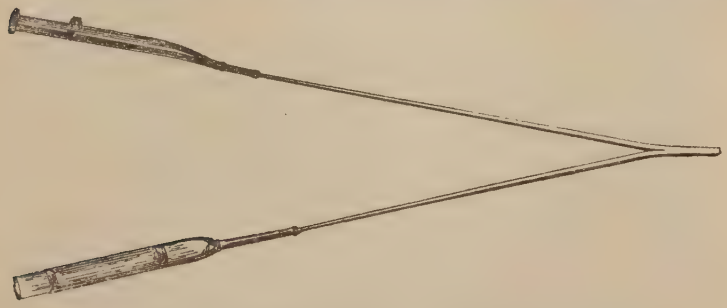

FIG. 7. Boyd's manipulating forceps for removing adults from cage.

supplied. The advantage of a lantern chimney is the ease of handling it.

Boyd(22) has devised a test tube on forceps, which facilitates the transfer of mosquitoes from cage to cage (text fig. 7). If

this mounted tube is used, the bottom of the chimney is covered with two pieces of rubber dam, or bathing cap. Each piece has a small slit in the middle and the two pieces are put together so that the slits are at right angles. This forms a valve opening through which the tube can be pushed to accomplish the removal or introduction of an insect. When the tube is withdrawn the valvelike slits close, preventing escape of mosquitoes from the chimney.

Two flat pieces of tin or of cardboard, each with a hole near one end, may be of use in transferring insects from one chimney to another. They should be large enough, so that when part of the tin or cardboard is completely covering the base of the chimney, the end with the hole projects beyond. The gauze covering of the chimney is loosened and the chimney placed on the unperforated end of the piece of tin. The gauze can then be pulled away, the tin blocking the escape of the insects. The same procedure is followed for the other chimney. Then the two chimneys are placed end to end with the two pieces of tin intervening. By sliding these pieces of metal, the holes can be brought into apposition and the insects driven from one cage to the other. Such tins can also be used in transferring insects from a vial to a chimney, the vial being pushed through the hole (which should have a diameter only slightly larger than that of the vial). To replace the gauze, the chimney with its temporary tin bottom cover is placed on the flattened gauze. Then the tin is pulled away and the gauze fastened in place as before. With a little practice one becomes adept at manipulating the living insects.

Aëdes mosquitoes will mate in lantern chimneys, and it is relatively easy to propagate a colony in the laboratory. *Culex* mosquitoes require somewhat larger cages and can be propagated without much difficulty in cages measuring 1 by 0.5 by 0.5 meter, but it is more difficult to propagate anophelines in captivity. The most successful experiments have been made by Boyd.(23) Occasionally in the Tropics freshly hatched anophelines will mate in a small cage, and viable eggs will result; but this is exceptional. Apparently a very large cage with carefully controlled water and food supplies is required. The cage should be large enough to accommodate a carabao or even several animals and some nearly natural breeding places, such as a section of a stream, or a small pool. We have not had success in colonizing anophelines in captivity in the Philippines in a cage measuring about 6 by 6 by 10 meters.

To obtain eggs for study, fully gravid females should be selected and placed in a small cage, such as an oil cylinder glass or a lantern chimney, over water. Christophers(5) suggests floating in the water a large but thin paraffin-coated cork ring slightly smaller in circumference than the water container and held up by the meniscus. This greatly facilitates the microscopical examination of the ova in situ. A piece of paper or a stick in the cage will give the mosquito a foothold. (See also above under the discussion of ova.)

PRESERVING AND MOUNTING ADULT ANOPHELINES (PLATE 6, FIG 2; TEXT FIG. 8)

There are many ways of preserving and mounting adult anophelines. The following account closely follows Boyd.(4)

Unmounted material can be kept either in the original pill boxes or transferred to test tubes. The former should be stowed in tight tin boxes, in which is kept a small dish of naphthalene and phenol to guard against insects and molds. If test tubes are used, they are prepared by pouring about 1 cubic centimeter of melted naphthalene into the bottom. This is covered by a small pledget of cotton. A layer of five or six mosquitoes is loosely arranged on this cotton, then another pledget of cotton is added, care being taken not to crush, but only to secure, the first mosquitoes. Alternate layers of mosquitoes and cotton are inserted until the tube is filled.

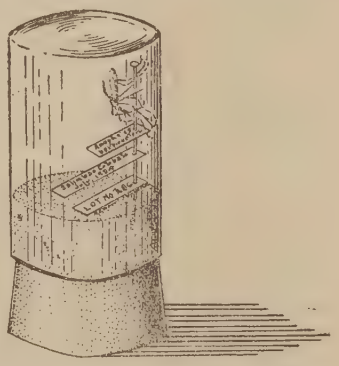

FIG. 8. One method of mounting an adult mosquito inside a glass vial.

Specimens which have been stored and are dry may be pinned for mounting if they are first softened. Several layers of moistened filter paper are placed in the bottom of a dish having a tightly fitting cover. The dried mosquitoes are spread on this paper and the cover replaced. After a few hours the insects can be mounted with little danger of breaking the appendages.

Pinned mounts are best made with freshly killed anophelines. When the mosquitoes are first exposed to chloroform vapor, the wings temporarily expand to a position at right angles to the body. MacGregor recommends that they be pinned immediately

upon becoming stupefied, thereafter being returned to the chloroform vapor until killed. Very fine pins 0.5 inch long, known by the German name "Minutenadeln," are best for insertion in the mosquito. These are without heads. Two methods of pinning may be followed.

(1) Expose the ventral surface of the specimen, grasp a pin with the pinning forceps, and insert the pin point into the ventral thoracic wall between the legs, gently pushing until the point just emerges from the dorsum of the thorax. With the pin still grasped by forceps, its base is forced into and put through a small oblong piece of pith or cork near one end of the latter. A No. 2 full-length mounting pin is forced through the opposite end of the piece of pith or cork, until the latter is fixed at about the middle of the pin. The mounted specimens are best preserved in small vials, a separate vial being devoted to each mounted specimen. The heavy pin of the mount is forced into the inner surface of the stopper, in a slightly eccentric location. These vials are of clear, thin glass, with no constriction at the neck. A good size measures two inches in length by one inch in diameter. The label of the specimen may be transfixed by the mounting pin before its insertion into the stopper, or pasted on the outside of the vial. The stoppers may be horizontally perforated about 3 mm. above the bottom, by a hole about three-eighths of an inch in diameter into which is poured melted naphthalene and phenol, which will quickly harden.

(2) Using slightly heavier and full-length pins from which the head has been snipped, the insect may be pinned by thrusting the point transversely through the thorax, then thrusting the cut end of the pin into the vial stopper. By this method the thoracic scales receive less damage. The vials are conveniently stored in pasteboard boxes divided into compartments, each compartment holding one vial.

Less valuable specimens may be pinned in ordinary insect-proof boxes, in which, however, they cannot be as readily examined.

Pinned specimens are not adapted to shipment through the mails. For this purpose specimens are best sent confined between layers of cotton in pasteboard pill boxes, a number of which can be shipped in a cylindrical mailing carton.

A good deal of trouble has arisen in the Tropics from oxidizing pins. The ordinary pins rust quickly. Silvered pins corrode in time and spoil the specimen. Nickel pins are best, but even these have not always proven satisfactory. It is now possible to buy stainless steel pins, but we have not yet tried these.

In our experience we have had good results putting no pin at all through the insect. We fasten the specimens by a very small drop of shellac to the tip of a small wedge of cardboard. This small piece of cardboard is then fastened to the cork with a No. 2 pin. The shellac is placed on the tip of the cardboard wedge, which is then carefully placed in contact with the

right side of the mosquito, which lies on its left side. To insure good adhesion the left side of the insect may then be pressed gently and carefully with a dissecting needle. This procedure, with a little practice, does no damage to important scales or bristles. It is possible to buy punches for cutting the cardboard wedges, but ordinary scissors may be used if necessary. Fairly long wedges are desirable when the insects are to be examined under high magnification. Practice is required in order to avoid damage to scales, wings, and legs, but when perfected the method gives excellent exposure for examination and the specimen does not deteriorate so rapidly as when pinned.

IDENTIFYING ANOPHELINE ADULTS

We have prepared and distributed a wall-chart key to the adult anophelines of the Philippines and have also presented a paper on the same subject.(3) The latter contains full descriptions of each species and also a discussion of the characters used for diagnosis.

A lens is required for the study of the specimens. A binocular entomological microscope is best, but an ordinary low-power monocular microscope can be used. In fact a strong doublet hand lens may be all that is required, although it will not give such good results as a microscope.

The examination can be made in reflected daylight or under artificial light. We have had good results using a focusing flashlight with three or four dry cells. This gives a powerful light which brings the bristles and scales into good relief. The light can be easily directed at any angle and does not give off as much heat as the ordinary microscope lamps (Plate 6, fig. 1).

With the specimen in good light under the microscope, the examiner must first be sure that it is a mosquito, and then, having decided that it is or is not an anopheline, note the sex. These three initial diagnoses are fundamental, and the following practical notes may be helpful:

HOW TO RECOGNIZE THE SPECIMEN AS A MOSQUITO

The following indicates that the place of mosquitoes among the insects is as follows: Phylum Insecta; order Diptera; suborder Nematocera; family Culicidæ; subfamily 1. Dixinæ; subfamily 2. Chaoborinæ; subfamily 3. Culicinæ (the true mosquitoes).

The word "diptera" is from the Greek meaning "with two wings." Therefore, the first criterion is that the specimen has two wings (and six legs).

The word "nematocera" is from the Greek meaning, loosely, "threadlike antenna." The Nematocera are small midgelike flies, with well-differentiated head, thorax, and abdomen, the last clearly segmented. The head bears two threadlike antennæ, which have from six to sixteen segments and are usually covered with fine, long hairs.

Next, to separate the Culicidæ from the other families in the Nematocera we note that all Culicidæ have a proboscis, long or short, and (except the Dixinæ) all have a dense fringe of scales which project backward along the posterior margin of the wings. Moreover, the delicate membranous wings always carry a complete venation as shown in text fig. 3 of our key.(3) If a given specimen does not have the posterior fringe of wing scales and the typical venation, it is not a mosquito.

Finally, to separate the subfamily Culicinæ, or true mosquitoes, from the other Culicidæ, note that all Culicinæ, or mosquitoes, have a long proboscis, which about equals the combined length of the head and the thorax.

HOW TO RECOGNIZE AN ANOPHELINE MOSQUITO

The Culicinæ are divided into three tribes; namely, Anophelini, Megarhinini, and Culicini. Edwards(24) gives the following key to these tribes:

Key to the tribes of Culicinæ.

1. Abdomen without scales, or at least with the sternites largely bare. Anophelini.
   Abdomen with both tergites and sternites completely clothed with scales. 2.
2. Proboscis rigid, outer half more slender and bent backwards. Megarhinini.
   Probscis more flexible, of uniform thickness (unless swollen at tip), outer half not bent..... Culicini.

In our key to local adult anophelines we have mentioned other characters that may be of assistance in separating the tribe Anophelini.

HOW TO IDENTIFY THE SEX

Most male mosquitoes can be recognized at once by their "whiskered" appearance. Their antennæ have many long hairs, which give a distinctly plumed appearance easily noted at a glance. The female antennæ are sparsely ornamented. There are a few mosquitoes (none of the Philippine *Anopheles*) in which the male and the female antennæ are similar. The ques-

tion can always be settled by examination of the terminalia or the tip of the abdomen under a strong hand lens.

The male terminalia have a somewhat broadened appearance. There are two conspicuous hooklike claspers which cannot be mistaken. The female genitalia are not conspicuous. The tip of the abdomen is more pointed and there are seen two small papillæ, called cerci, which project either vertically or longitudinally (text fig. 9).

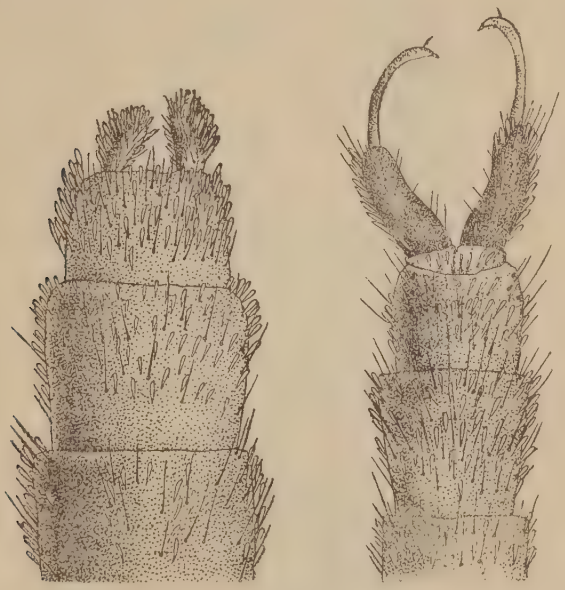

FIG. 9. Tip of abdomen of anopheline adults, male on right, female on left, showing distinctive characters.

Once the specimen in question is known to be an anopheline mosquito, it may be examined systematically with our key(3) at hand. When by means of the key a probable diagnosis of species is arrived at, the descriptive text referring to that species may be consulted to check in detail the various characters. When the drawings given in the corresponding plate are consulted, in most cases there will remain no doubt as to the identification of the specimen.

In all doubtful cases the specimen should be sent to the Health Service or to the Bureau of Science for further study, together with as many details about habitat as possible. Dried mounted

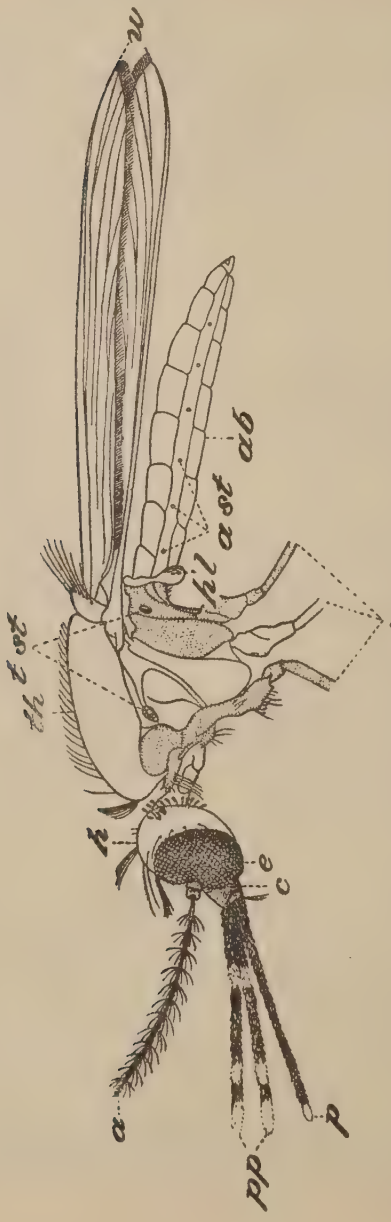

FIG. 10. Adult mosquito, showing, *p*, proboscis; *pp*, palpa; *a*, antenna; *c*, clypeus; *e*, eye; *h*, head; *hl*, haltere; *th*, thorax; *tst*, thoracic stigmas; *ab*, abdomen; *ast*, abdominal stigmata; *w*, wings; *ll*, stumps of legs. (After Nuttall and Shipley.)

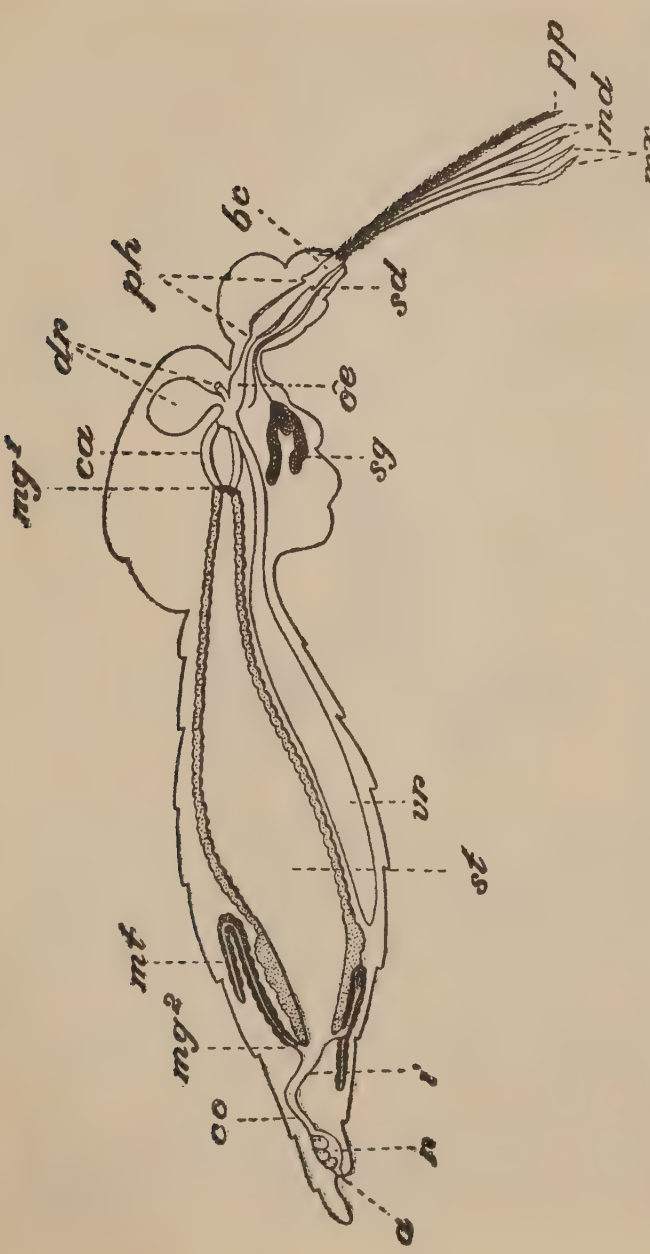

FIG. 11. Gross internal anatomy of mosquito, showing, *pp*, palp; *md*, mandibles; *mx*, maxillæ; *bc*, buccal cavity; *ph*, pharynx; *sd*, salivary duct; *oe*, œsophagus; *sg*, salivary glands; *dr*, dorsal reservoirs; *vr*, ventral reservoir; *ca*, cæca; mg^1, midgut begins; mg^2, midgut ends; *st*, stomach; *mt*, Malpighian tube; *i*, ileum; *co*, colon; *r*, rectum; *a*, anus. (After Nuttall and Shipley.)

mosquitoes usually do not have their abdomen in the right position for examination. In such cases the entire abdomen is cut off and heated, first in a 10 per cent potassium hydroxide solution, and subsequently in distilled water. This process renders the organ in excellent condition for examination. The hairs and scales are not removed, the whole abdomen is transparent, and even the color of the scales is retained. For future reference such specimens may be preserved in formalin-borax solution or weak alcohol, and kept in a properly labeled small vial.

DISSECTING ADULT MOSQUITOES (TEXT FIG. 12)

Gut.—There are many ways of dissecting out the gut of a mosquito, and each technician has his own ideas as to which is best. The following description is based on Boyd's(4) directions.

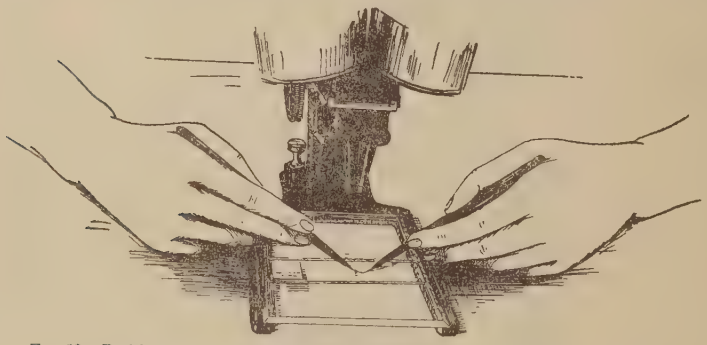

FIG. 12. Position of microscope, hands, slide, needles, and insect during dissection.

The specimens to be dissected are killed one at a time as needed, with the use of chloroform vapor. It is much better not to attempt to use those that have a gut distended with blood, but to dissect only those that have fully digested their last blood meal. Careful note should be taken of the species, as this is very important. In the Philippines, so far, only *A. minimus* var. *flavirostris* and *A. maculatus* have been found infected in nature. While the insect is held with forceps, which grasp the palps and proboscis, the wings and legs are cut off close to the body.

The mosquito is placed on a glass slide in a drop of physiological saline solution, which has been deeply tinted with methylene blue, and arranged with dissecting needless so that the dorsum of the insect is away from the dissector and the tip of the

abdomen at the right. Under a low-power lens the insect is held firmly by means of the straight needle thrust into the thorax near the abdomen. With the curved needle cuts are made into the seventh or eighth abdominal segment, both dorsally and ventrally. Then the last segment of the abdomen is transfixed with the tip of the curved needle, and the viscera attached to this abdominal tip are drawn out. The foregut usually breaks, and it is easy to draw out the viscera into the saline solution. (Some dissectors prefer to cut the abdomen away from the thorax before dissecting out the gut.)

The body of the mosquito is put to one side and the gut cleaned by gently cutting off the Malpighian tubes and other structures posterior to them. The clean gut or "stomach" is transferred by means of the needle to a drop of untinted saline solution on a clean slide and protected by a cover slip. The slide is gently heated over an alcohol lamp so that the heat will expand the gases inside the gut and so extend the walls, making them thinner.

The preparation should next be searched under high power for oöcysts. These cysts vary in size from about 6 to 50 microns. They are approximately spherical and appear a little more opaque than the gut cells. Because they are attached to the stomach wall they are usually distinct from yeast cells or similar bodies contained within the cavity of the stomach. One of the most useful criteria for the diagnosis of an oöcyst is the clump or chain of brown or black pigment granules which are always present in all but the very large oöcysts. These very large cysts are mature and striated with innumerable sporozoites. Such large cysts may be ruptured by slight pressure and the sporozoites, as they escape, are recognized by their morphology, as noted below. A small cyst should never be diagnosed as a malarial oöcyst unless it is definitely pigmented and is seen to be attached to the stomach wall. Sometimes, if the stomach is rolled a little, by pressing a needle on the cover slip, a doubtful body may be seen not to be attached to the wall.

Fat droplets and immature ova on or under the stomach, yeast cells in the stomach cavity, and occasionally other bodies may simulate oöcysts; but if the cyst is definitely attached to the stomach wall, and if it certainly contains either pigment granules or recognizable sporozoites, there can be little confusion. Small oöcysts up to 7 or 8 μ have no definite cyst wall. A single stomach may show from one to more than one hundred oöcysts (Plate 7).

Salivary glands.—What remains of the mosquito from which the gut was dissected should be turned so that the head and thorax project towards the right. The specimen is held in place by transfixing the posterior part of the thorax with the straight needle held in the left hand and the head carefully cut off with one clean, quick cut with an edged needle. The dull side of a needle held in the right hand is pressed gently on the middle of the thorax, forcing a small amount of tissue out at the neck end. Under a lens this tissue is cut off and gently teased apart, the examiner being alert to recognize the two blue-stained and trilobed salivary glands, which will show up clearly among the unstained muscle fibers. Then the glands are transferred to a drop of untinted saline solution on a clean slide, covered with a cover glass, and examined for sporozoites (Plate 8, figs. 1 and 2).

The thin fusiform sporozoites are usually easily recognized. They are either straight or slightly curved and average 12 to 14 μ in length. When alive they have a slow, sinuous movement. They must be distinguished from such artifacts as rod-shaped bacilli or crystals. It is always a good plan to stain them with Wright's or Giemsa's stain. Each sporozoite will show blue or violet cytoplasm and red chromatin. To stain, the cover is carefully lifted and inverted on a dry slide on a tiny drop of balsam. The balsam holds the cover slip in position while the preparation is dried, fixed, and stained. For description and methods of preserving these glands, see Manalang.(25, 26)

It is quite possible to dissect out the glands quickly and easily simply by cutting off the head, as the mosquito lies on its side in a drop of saline solution, and pressing gently on the pleura of the thorax, expressing the glands, often well isolated. It is not even necessary to remove the legs or wings, after a little practice. We have had good results by this simple technic when we have had many specimens to examine in a short time. But much better preparations are secured by the method outlined above.

Salivary-gland dissections may be dried on the slide, fixed, and subsequently stained with Wright's or Giemsa's stain, and sent to a central laboratory for confirmation of diagnosis.

The dissection of salivary glands of *Culex* mosquitoes is more difficult and requires additional practice.

Terminalia.—The dissection and preparation of the male terminalia are not easy and require a great deal of practice.

After the terminal segment has been cut off it is heated in a 10 per cent solution of potassium hydroxide. Care is taken not to heat it beyond the first steaming point, the correct temperature being about 60° C., such that the finger can just bear to touch the dish. Overheating makes such delicate parts as the leaflets of the phallosome very transparent so that it cannot be determined whether the thinner edges are serrated or not.

The phallosome is severed from its basal connections with the coxites, the theca is cut short, after which the apical portion, together with the leaflets, is transferred to a clean slide, with a tiny drop of Gater's fluid. The material is carefully immersed in the fluid and watched through a binocular while a cover slip is put in place. With a stout needle the cover glass is pressed down firmly when the phallosome is in the right position. The pressure helps to spread the leaflets nicely on either side without the need of cutting the theca into two longitudinal halves. The mounting fluid should be so little as to spread barely to the edges of the cover slip when pressed. Additional fluid may be added to make up the shrinkage after drying. Care should be exercised to use perfectly flat cover slips, as those which are a little convex spoil the best dissections by not staying in position when pressure is removed.

The isolation of the harpago is best done by pressing the outer side of the coxite with a needle directed inwardly at an angle of about 45°. This tilts the coxite a little and exposes the harpago to the best advantage for dissection.

SUMMARY

The technic of handling mosquitoes requires patience and practice, but it is not, after all, very difficult. It is essential that one have available a small library. If funds are limited, we should suggest as a minimum the following list:

Handbooks: Boyd's Malariology(4) and MacGregor's Mosquito Surveys.(27) Reprints: King,(12, a, b, c) Manalang,(10, 25, 26) and Russell and Baisas.(1, 2, 3) Wall charts: Key to Larvæ of Philippine Anopheles and Key to Adult Philippine Anopheles, published by Malaria Investigations and obtainable through the Bureau of Health.

The investigation of Philippine mosquitoes is a matter of importance, and so little has been done that it is a fruitful field for research.

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ILLUSTRATIONS

PLATE 1

FIG. 1. Collecting larvæ, using dipper on the end of a rattan cane, making wide sweeps of surface water.
2. Collecting larvæ, dipper being pushed against a bank so that water flows into it from among the roots of the vegetation.
3. Carrying case for larva-collecting bottles.

PLATE 2

FIG. 1. Larva-collecting outfit. Three types of dipper, strong rattan cane, collecting pipettes, half-pint collecting bottles, knapsack carrying case, and rubber boots.
2. Rearing outfit for larvæ. Collecting bottle, enamel pans, glassware, pupal tubes, and pipettes.

PLATE 3

FIG. 1. Collecting adults. Showing a typical *Anopheles minimus* var. *flavirostris* breeding place and a collector using glass and rubber tubing, taking adults from a daytime shelter in small caves formed by an undercut stream bank.
2. Collecting adults with a glass-and-rubber, suction, catching tube. Natural daytime shelter.
3. Catching adults with a small vial. Natural daytime shelter.

PLATE 4

FIG. 1. Anopheles adult trap, at Iwahig, suitable for carabao bait, distant view.
2. *Anopheles* adult trap suitable for carabao bait, near view.
3. Earth-lined *Anopheles* adult trap, with cover held up.

PLATE 5

FIG. 1. Catching of adult *Anopheles* on carabaos at night. (Photograph taken in daytime.)
2. Lantern chimney, tin cover for transferring mosquitoes, and wire cage with cloth-sleeve entrance. Adults that may be infected are transferred from vials to lantern-chimney cage inside this larger cage.

PLATE 6

FIG. 1. Flashlight used as a source of illumination for the examination of adult mosquitoes. Method of placing a specimen for examination and the type of binocular microscope suitable for this purpose. A simple stand to hold the flashlight can easily be made.
2. Method of mounting an adult mosquito in a small drop of shellac, as explained in the text.

PLATE 7

Numerous oöcysts of malaria on the stomach wall of *Anopheles minimus* var. *flavirostris*.

PLATE 8

FIG. 1. A mature oöcyst under higher magnification, showing sporozoites. [From Manalang.(25)]

2. Sporozoites from a ruptured salivary gland; × 1,000. Stained. [From Manalang.(25)]

3. Sporozoites from a ruptured salivary gland, in saline solution; × 1,000. Unstained. [From Manalang.(26)]

TEXT FIGURES

FIG. 1. *Culex* egg raft.

2. Two types of eggs of *Anopheles minimus* var. *flavirostris*. (Drawn by Andres Nono.)

3. Special type of dipper.

4. Larva shipping bottle.

5. Vial for shipping living adults.

6. Lantern-chimney cage with manipulating tin. Dotted lines show position of base of chimney when adults are being inserted or withdrawn.

7. Boyd's manipulating forceps for removing adults from cage.

8. One method of mounting an adult mosquito inside a glass vial.

9. Tip of abdomen of anopheline adults, male on right, female on left, showing distinctive characters.

10. Adult mosquito, showing, *p*, proboscis; *pp*, palps; *a*, antenna, *c*, clypeus; *e*, eye; *h*, head; *hl*, haltere; *th*, thorax; *tst*, thoracic stigmas; *ab*, abdomen; *ast*, abdominal stigmata; *w*, wings; *ll*, stumps of legs. (After Nuttall and Shipley.)

11. Gross internal anatomy of mosquito, showing, *pp*, palp; *md*, mandibles; *mx*, maxillæ; *bc*, buccal cavity; *ph*, pharynx; *sd*, salivary duct; *œ*, œsophagus; *sg*, salivary glands; *dr*, dorsal reservoirs; *vr*, ventral reservoir; *ca*, cæca; mg^1, midgut begins; mg^2, midgut ends; *st*, stomach; *mt*, Malpighian tube; *i*, ileum; *co*, colon; *r*, rectum; *a*, anus. (After Nuttall and Shipley.)

12. Position of microscope, hands, slide, needles, and insect during dissection.

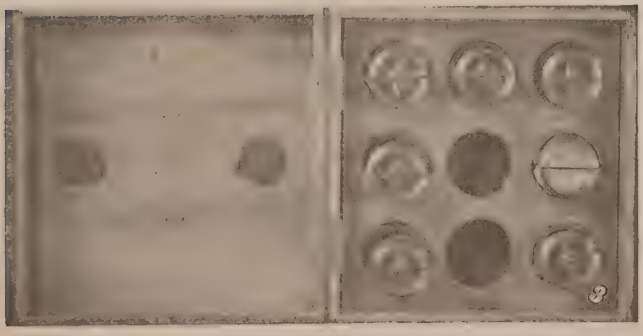

PLATE 1.

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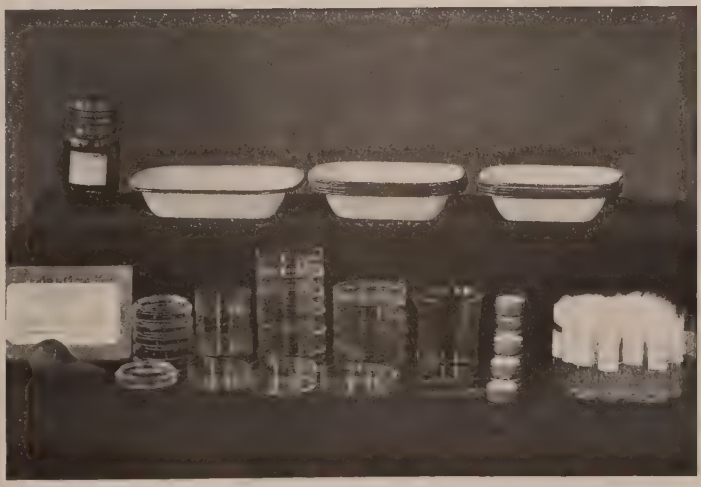

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PLATE 2.

PLATE 3.

PLATE 4.

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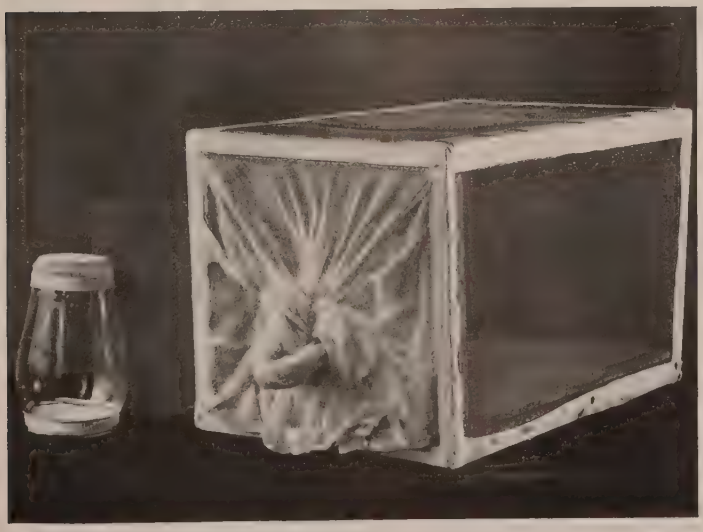

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PLATE 5.

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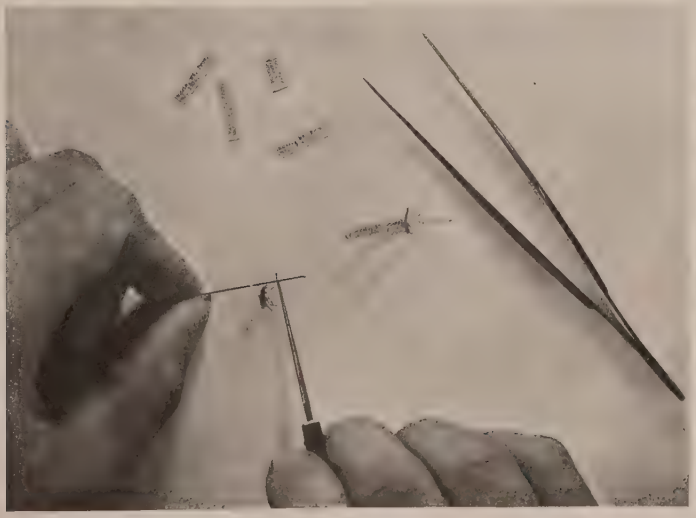

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PLATE 6.

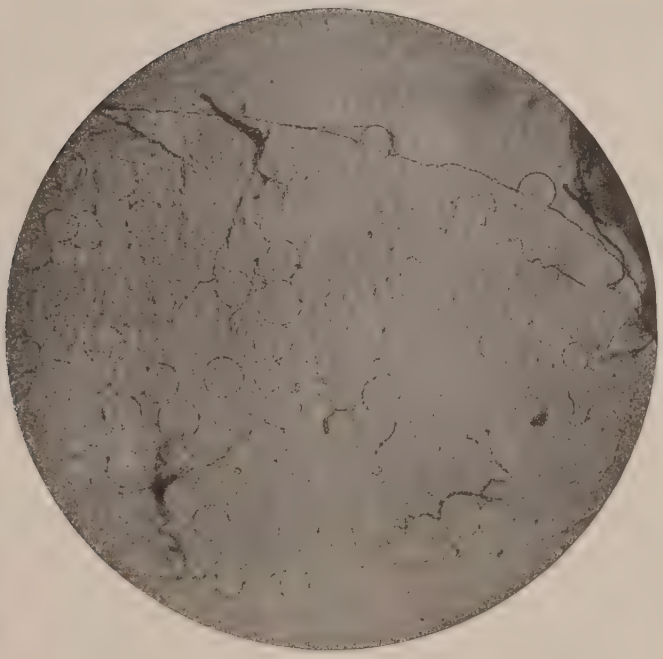
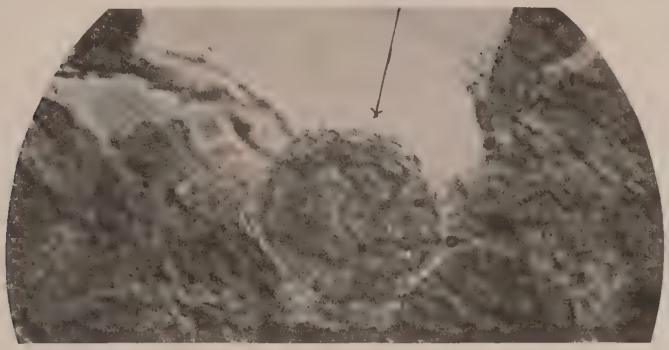


PLATE 7.



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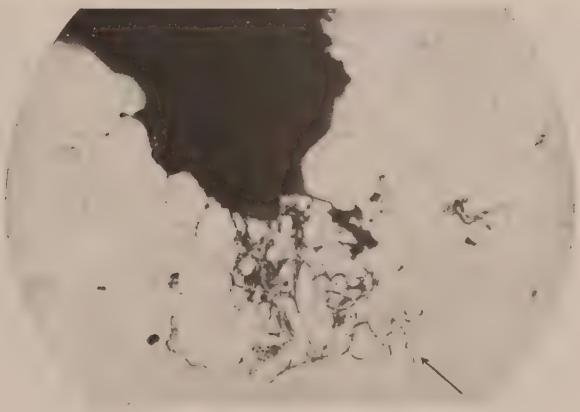

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PLATE 8.

EFFECTS OF CHLORINATED LIME IN LETHAL CONCENTRATIONS ON ENTAMŒBA HISTOLYTICA CYSTS [1]

By Eusebio Y. Garcia

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ONE PLATE

In an experiment of this nature it is important to establish a safe and satisfactory criterion for determining the viability of the cysts. A review of the literature on the subject reveals that there is no standard criterion by which to determine whether a cyst is dead or living. Various authorities who have worked on the determination of the longevity of *Entamoeba histolytica* cysts in vitro have reported various results with widely varying significant differences, probably because different criteria were used in judging the viability of the cysts.

Kuenen and Swellengrebel (1913), working on the hypothesis that dead cysts stain with a weak solution of eosin in water (1:1,000) and that living cysts do not, found that all the cysts in fæces begin to degenerate in from thirteen to twenty-nine days. The reliability of this result was later discounted by Yorke and Adams (1926), who gave as a proof of their contention the nonexcystation of cysts that they believed to be living in the Locke-egg-serum medium of Boeck and Drbohlav (1925). Penfold, Woodcock, and Drew (1916), determining the viability of the cysts by their power to excyst in the presence of liquor pepticus, stated that although they were able to keep cysts in slow-running water for fifteen days, some certainly were alive at the end of this period. Wenyon and O'Connor (1917), employing the eosin test for viability, claimed that cysts will survive for over thirty days in water. Dobell (1919), cited by Yorke and Adams (1926), employing the eosin test supplemented by morphological observations, found that the maximum viability of cysts in water is five weeks, and that they may be found dead at the end of a week in fæces. Boeck (1921), also

[1] Read before the Third Philippine Science Convention, held in Manila February 28, 1935.

using the eosin test supplemented by morphological observations, stated that *E. histolytica* cysts were viable at the end of one hundred fifty-three days in water. This very high figure led some investigators to doubt the accuracy of such results, and in 1932 Wight and Wight, after observing the viability of the cysts by actual culture, claimed that Boeck never proved that after this long period (153 days) the cysts that he believed survived were capable of perpetuating the species. Long after the cysts are moribund or dead, the wall may retain impermeability for certain dyes. Sellards and Theiler (1924) modified the method of Kuenen and Swellengrebel for determining the viability of the cysts by infecting kittens by intrarectal injections of the cysts instead of per os, and to their disappointment they failed to produce infection even with the use of quite fresh material. Wight and Wight (1932) seemed able to prove that the conclusion of Yorke and Adams (1926), based on cultural methods, were entirely inadequate, and that Dobell's figure (37 days plus), based on the eosin test seemed to be nearer the truth. The same authors claimed that the majority of the dead cysts of Yorke and Adams on Locke-egg-serum medium were still viable when subcultures were made in the same medium. From the latter observation we may infer that cultural methods, no matter how favorable the medium, are not a satisfactory criterion for determining the viability of cysts, because all cysts that have been exposed to the action of one chemical or drug, or any test solution or test medium, will not excyst at the same time, as not all of them have the same resistance. This inference seems to be borne out by the fact that the same authors (Wight and Wight, 1932) found that the preëxcysting forms in Locke-egg-serum medium are similar to dead cysts and vice versa.

From these contradictory, not to say confusing, reports, it seems clear that the results of the different investigators are much at variance. In the writer's opinion these varying results are due to the lack of a standard criterion for judging the viability of the cysts. The writer is carrying on experiments along similar lines but using for determining the viability of cysts an entirely different criterion, based on the principle of cell necrobiosis, which, in the writer's opinion, is more reliable than the criteria used hitherto. Cystologists and physiologists have demonstrated that the nucleus as a whole is the governing body of the cell, and without it the cell is considered dead and beyond resuscitation. No one would deny that the cyst is in the

dormant and resistant state of a living cell which has met unfavorable conditions. That being the case, the nucleus is the most important structure of the cyst. If parts of the protoplasm of a cell are lost, leaving only a very thin rim around the nucleus, as long as the nucleus remains intact the cell is still living, and able to regenerate and to perform its physiological functions. If the nucleus is lost, the whole cell is lost. In other words, a dead cyst may be defined as one whose nucleus or nuclei have been destroyed or have disappeared, and whose cytoplasm is shrunken, coarsely granular, and vacuolated. Cysts that do not answer to this definition are not considered dead.

An opportunity to test the reliability and usefulness of a criterion for the determination of cyst viability presented itself when Dr. A. Ocampo, of the Philippine General Hospital, supplied the writer with fæces from a confirmed case of entamœbic dysentery. There could be no doubt that the patient was suffering from chronic amœbiasis. The cysts present in the fæces answered all the morphological descriptions of typical *E. histolytica* cysts; and, therefore, there seems to be no doubt that *E. histolytica* cysts were used in this study.

In view of the fact that the results of Wenyon and O'Connor (1926), that chlorinated lime tabloids as used for water sterilization had no action on *E. histolytica* cysts, seem unreliable, because their findings were based on the use of eosin as a test for viability; and that the results of Yorke and Adams (1926) are subject to the same criticism, as proven by Wight and Wight (1932), the writer felt justified in making further observations on the action of chlorinated lime on *E. histolytica* cysts. The writer was also influenced by his interest to determine the lowest concentrations lethal to the cysts, because he believes this is important from the standpoint of water sterilization by the use of chlorinated lime, which is now being used in large and small filtration plants and in the sterilization of swimming pools.

In order to forestall errors in this experiment an entirely different line of approach was followed. The chemistry of hypochlorite of lime ($CaOCl_2$), especially its rate of solubility in free chlorine water, was studied, and it was found that 1 gram of the powder will completely dissolve in forty-five minutes in 100 cubic centimeters of water without stirring, and in thirty minutes with stirring. These findings approximate those of Buswell (1923). According to Buswell $CaOCl_2$ is not very soluble

TABLE 1.—*Percentages of different types of Entamoeba histolytica cysts observed in different dilutions of lime at different exposures.*

Hypochlorite of lime dilutions (5 cc).	Cysts observed.	Morphologic descriptions.												Control. Original fæces with 96 per cent = 4 nuclei, 4 per cent = 2 nuclei.
		30 min.	Type.	Per cent.	45 min.	Type.	Per cent.	1 hr.	Type.	Per cent.	1 hr. 15 min.	Type.	Per cent.	
1 : 100	100	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	98	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm very much shrunken and vacuolated.	IV	100							No change.
		Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and vacuolated; chromatoid bodies fragmented.	I	2										

1	1,000	100	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	89	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm shrunken and vacuolated.	IV	95	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm very much shrunken and vacuolated.	IV	100	No change
			Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and vacuolated; chromatoid bodies fragmented.	I	11	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	5				

TABLE 1.—*Percentages of different types of Entamoeba histolytica cysts observed in different dilutions of lime at different exposures*—Continued.

Hypochlorite of lime dilutions (5 cc).	Cysts observed	Morphologic descriptions.											Control. Original fæces with 96 per cent =4 nuclei, 4 per cent =2 nuclei.
		30 min.	Type.	Per cent.	45 min.	Type.	Per cent.	1 hr.	Type.	Per cent.	1 hr. 15 min.	Type.	Per cent.	
1 : 10,000	100	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	86	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm shrunken and vacuolated.	IV	94	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm shrunken and vacuolated.	IV	100	----------	------	------	
		Two nuclei distorted and two broken; protoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II	10	Two nuclei distorted; protoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II	4	----------	------	------	----------	------	------	

1 : 10,000	100	Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated.	I		4	Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	I						No change.
		Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken and vacuolated; chromatoid bodies fragmented.	III	80	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm shrunken and vacuolated.	IV	90	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm shrunken and vacuolated.	IV	98	Distorted and broken; four nuclei disappeared; chromatoid bodies disappeared; cytoplasm shrunken and vacuolated.	IV	100
1 : 100,000	100	Two nuclei distorted and two broken; cytoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II	15	Two nuclei distorted and two broken; protoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II	6	Two nuclei distorted and two broken; protoplasm shrunken and vacuolated; chromatoid bodies fragmented.	II	2			

TABLE 1.—*Percentages of different types of Entamoeba histolytica cysts observed in different dilutions of lime at different exposures*—Continued.

Hypochlorite of lime dilutions (5 cc).	Cysts observed	Morphologic descriptions.												Control. Original fæces with 96 per cent= 4 nuclei, 4 per cent= 2 nuclei.
		30 min.	Type.	Per cent.	45 min.	Type.	Per cent.	1 hr.	Type.	Per cent.	1 hr. 15 min.	Type.	Per cent.	
1 : 100,000	100	Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated.	I	5	Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated.	I	4	----------	----------	----------	----------	----------	----------	No change.
1 : 1,000,000	100	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	41	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	56	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	72	Four nuclei distorted and broken; karyosome disappeared; cytoplasm coarsely granular, shrunken, and vacuolated; chromatoid bodies fragmented.	III	73	

1 : 1,000,000	100	Two nuclei distorted and two broken; cytoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II	1	39	Two nuclei distorted and two broken; cytoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II		24	Two nuclei distorted and two broken; cytoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II	1	20	Two nuclei distorted and two broken; cytoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II	1	20	No change.
		Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated.			20	One nucleus broken and three distorted; cytoplasm shrunken and slightly vacuolated; chromatoid bodies fragmented.	II		10	Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated.			8	Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated.			7	
						Four nuclei distorted; karyosome disappeared; cytoplasm shrunken and slightly vacuolated.	I		10									

and is ordinarily fed as 1 or 2 per cent suspension. This is important to know, because it will enable us to predetermine the strength of the dilutions to be used to test the action of this chemical on the cysts. Judging from the results of Wenyon and O'Connor (1926) and Yorke and Adams (1926) it seems reasonable to suppose that this important fact had been ignored by these authors.

Another important feature in this work was the use of natural fæces instead of washed and concentrated suspensions, as done by Yorke and Adams (1926). While the use of the latter is more ideal than the use of the former, the writer believes, as he points out in his discussion of the results, that the presence of nitrogenous organic matter in the fæces is most necessary in the action of $CaOCl_2$ on the cysts. Some chemical substances are formed in the interaction between calcium hypochlorite and nitrogenous organic matter, and these have a synergistic killing effect on *E. histolytica* cysts. The presence of nitrogenous organic matter in the fæces is always certain, because bacterial decomposition of proteins takes place in the fæces and the bacteria are capable of deamidizing these nitrogenous substances into free ammonia (NH_3) and fatty acids.

METHODS

Sixteen-hour-old fæces of jellylike consistency were stirred thoroughly with a platinum loop 5 millimeters in diameter. One loopful of the fæcal emulsion was spread uniformly on a large slide, covered with a large cover glass, and sealed with paraffin. A total count of the cysts was made under a high-power lens after the manner of Stoll's egg-counting technic; that is, by the use of a square fenestra attached to the eye piece and a cliquer. Counts were made on several such preparations, and it was found that each loopful contained an average of fifty-six cysts. The types of cysts present were 96 per cent quadrinucleates and 4 per cent binucleates (Plate 1, figs. 1 and 2).

After the average number of cysts per loopful was determined, 1 per cent solution of calcium hypochlorite ($CaOCl_2$) in distilled water was prepared by shaking the preparation vigorously in a tightly stoppered flask until the $CaOCl_2$ was completely dissolved. From this original solution varying dilutions up to 1 : 1,000,000 were made. Five cubic centimeters of each of these test solutions were placed in a tightly stoppered test tube and the series arranged in sequence in a test-tube rack. Four loop-

fuls of the fæces were inoculated to each test tube containing the test solution. By means of a capillary pipette two drops were taken from the bottom of each test solution after thirty minutes and spurted on a slide, covered with a cover glass, and examined under an oil-immersion objective. A careful study was made of the internal organization of each cyst that came under observation, and the ratio between living cysts and the dead ones determined by use of the aforementioned criterion. This procedure was repeated in another series, in which washed and concentrated suspensions of *E. histolytica* cysts were used instead of fæces containing cysts. The results are shown in Tables 1 and 2.

TABLE 2.—*Percentages of different types of washed Entamœba hystolytica cysts that were observed in different dilutions of hypochlorite of lime.*

Hypochlorite of lime dilutions (5 cc).	Types of *Entamoeba histolytica* cysts.								Control. Concentrated and washed *E. histolytica* cysts with 96 per cent = 4 nuclei, 4 per cent = 2 nuclei.
	30 min.	Per cent.	45 min.	Per cent.	1 hr.	Per cent.	1 hr. 15 min.	Per cent.	
1 : 100	III	62	IV	44	IV	84	IV	100	
	II	20	III	42	III	16	------	------	
	I	18	I	14	------	------	------	------	
1 : 1,000	III	53	III	60	III	72	IV	51	
	II	26	II	22	II	28	III	49	
	I	21	I	18	------	------	------	------	
1 : 10,000	III	44	III	53	III	64	IV	42	
	II	28	II	33	II	23	III	58	No change.
	I	28	I	14	I	13	------	------	
1 : 100,000	II	64	III	26	III	32	III	33	
	I	36	II	40	II	47	II	46	
	------	------	I	34	I	21	------	21	
1 : 1,000,000	II	43	II	48	III	27	III	29	
	I	57	I	52	II	51	II	50	
	------	------	------	------	I	22	I	21	

DISCUSSION

Table 1 shows that while striking changes could be noted in the structure of the cysts exposed to different tests solutions, no changes at all occurred in those in the control tubes. The highest dilution of calcium hypochlorite ($CaOCl_2$) that will kill *E. histolytica* cysts after one hour fifteen minutes exposure is 1 : 100,000. This concentration, when reduced to parts per million, is equal to 10 parts per million; or, when expressed in chlorine, it is equal to 3.5 parts per million, because the available

chlorine present in the solution is 35 per cent. Aside from these striking changes, several types of degenerative changes in the cysts were found, not only in single test solutions but in all of them at different exposures. The results indicate beyond a doubt that calcium hypochlorite ($CaOCl_2$) really exerts a deleterious influence on *E. histolytica* cysts.

The efficiency of $CaOCl_2$ in killing *E. histolytica* cysts noted in this work is attributed by the writer to the fact that cysts were inoculated immediately after this chemical has been completely dissolved, allowing a maximum ionic action of the solution on the exposed cysts. It is presumed that either the hypochlorous (HClO) or chlorine (Cl) ions may penetrate the cyst wall and there act on the protoplasm of the cyst by oxidation. The chlorine (Cl_2) excess liberated by $CaOCl_2$ that does not act on cysts may by its addition and substitution reactions act on the decomposition products of protein, which are mostly nitrogenous organic compounds present in the fæces. Dakin et al. (1916) found that the combination of Cl_2 with nitrogenous organic matter forms strong antiseptic chloramines, which have stronger bactericidal action than chlorine, due to their greater solubility and penetrating power. They do not decompose spontaneously as does HClO and, therefore, their entire strength is maintained for a longer time. In this work greater action of $CaOCl_2$ was expected because the preparation that contained the cysts also contained organic matter.

By repeating the experiment of Yorke and Adams (1926) simultaneously with the first experiment, the writer found that only a 1 : 100 dilution of $CaOCl_2$ can kill *E. histolytica* cysts after one hour fifteen minutes exposure (Table 2). This result partly confirmed their conclusion in 1926 that only high concentrations of $CaOCl_2$ can kill *E. histolytica* cysts. It seems, however, that in the 1 : 100 dilution of $CaOCl_2$ the action of the chemical is not wholly due to Cl_2, but partly to traces of chloramines formed from the small quantity of nitrogenous organic matter which was left with the cysts after several washings and centrifugations. It is natural to expect, therefore, that in a concentrated fæcal emulsion this killing power may be tremendously multiplied, since it destroys cysts in a dilution as weak as 1 : 100,000.

APPLICATION OF CHLORINATED IODIZED SOLUTION

In order to find the lowest concentration of $CaOCl_2$ suitable for internal administration, the writer repeated the same procedure and made a series of dilutions of $CaOCl_2$ from 1: 1,000,000 to 5: 1,000,000, and to every 5 cubic centimeters of the test solution a 5 per cent solution of potassium iodide (KI) saturated with iodine was added to form a 0.5 per cent solution in the various concentrations of $CaOCl_2$. The fæcal material used in the previous experiment was utilized. To his surprise he found the same structural changes with slight deviations from those in Table 1 at different exposures. He found that the lowest concentration of $CaOCl_2$ solution with iodine that will kill *E. histolytica* cysts after one hour fifteen minutes is 4 : 1,000,000. The available chlorine (Cl_2) is about 35 per cent; so that, expressing this in parts per million of Cl_2, it is 1.4 parts per million with 0.5 per cent part of iodine (Table 3).

TABLE 3.—*Percentages of different types of Entamœba histolytica cysts observed in different dilutions of chlorinated iodized solutions.*

Hypochlorite solution with iodine in 0.5 per cent parts.	Types of *Entamoeba histolytica* cysts.								Control. Original fæces with 96 per cent = 4 nuclei, 4 per cent = 2 nuclei.
	30 min.	Per cent.	45 min.	Per cent.	1 hr.	Per cent.	1 hr. 15 min.	Per cent.	
1 : 1,000,000	III	42	III	58	III	73	III	75	
	II	38	II	32	II	20	II	19	
	I	20	I	10	I	8	I	6	
2 : 1,000,000	III	47	III	72	III	77	III	82	
	II	35	II	29	II	17	II	18	
	I	18	I	9	I	6	------	------	
3 : 1,000,000	III	58	III	76	III	84	III	91	No change.
	II	30	II	25	II	13	II	9	
	I	17	I	7	I	3	------	------	
4 : 1,000,000	III	81	III	92	IV	96	IV	100	
	II	14	II	6	III	4	------	------	
	I	5	I	2	------	------	------	------	
5 : 1,000,000	III	90	IV	97	IV	100	------	------	
	II	7	III	3	------	------	------	------	
	I	3	------	------	------	------	------	------	

The mechanism of action of these two chemicals seems to depend on the production of two nascent halogens of chlorine (Cl_2) and iodine (I_2), which are believed to have strong pene-

trating power through the cyst walls. The nascent Cl_2 of $CaOCl_2$ liberates I_2 from KI, and this nascent iodine (I_2) plus free I_2 and Cl_2 are perhaps enough to kill the cysts. We must not forget that the chloramines formed seem to act also on the cysts, and these help the two halogens in their killing power. The writer can think of no other explanation to account for this killing power.

The above results naturally suggest the possibility of using this solution in the treatment of the "cyst-carrier state," which Craig (1932) defined as a stage of amœbiasis where symptoms may be present or absent and, if present, mild and atypical, the condition being characterized by normal or frequent bowel movements with abundant cysts in the stool. It is true that a large percentage of the cysts in the colon are found in the lumen mixed with fæces, and a small percentage in the crypts of the mucosal folds. In the selection of the chemical to kill these cysts, the one that will act directly on the cysts and is, if possible, nonirritating, easily absorbed, excreted by the system, and effective in small doses should be chosen. Considering the concentration of calcium hypochlorite ($CaOCl_2$) and iodine in this mixture, 1.4 parts per million of available chlorine (Cl_2) is suitable for human use and 1 per cent of iodine is the average dose of the United States Pharmacopœia. In this solution the writer has only 0.5 per cent iodine, which is 50 per cent less than that permitted by the pharmacopœia and his experience as well as that of others shows that 0.5 per cent iodine in water enemata produces no toxic effects, except a mild irritation. It was shown also by Sollmann (1917) that iodine is easily absorbed in the colon and easily excreted by the system, especially by the salivary glands and kidneys. Furthermore, many drugs in the market contain iodine. Some of them, as summarized by Muhlenns (1921), are iodoxyquinoline sulphate, iodochloroxyquinoline, sodium iodoxyquinoline sulphonate (chinioform), iodochloroxyquineline (vioform), and the popular Yatren 105. While all these drugs contain iodine as the basic principle, and while various authors claim them to be efficacious, the writer believes that chlorinated iodized solution can be used with advantage, because of its low cost and easy preparation. Although the efficacy of this solution has not been tried in vivo, it certainly holds promise in the treatment of cyst carriers.

SUMMARY AND CONCLUSIONS

It may be inferred from the results of the foregoing experiments—

1. That the structural changes based on necrobiosis of the cell constitute an accurate and reliable criterion of the viability of *Entamœba histolytica* cysts.

2. That calcium hypochlorite ($CaOCl_2$) solution is destructive to *E. histolytica* cysts even in as low a concentration as 1: 100,000 or 3.5 parts per million of chlorine.

3. That the presence of nitrogenous organic matter enhances greatly the destructive power of calcium hypochlorite ($CaOCl_2$) to *E. histolytica* cysts.

4. That the concentration of 3.5 parts per million of chlorine (Cl_2), which is effective in killing *E. histolytica* cysts, is not suited for human use and cannot be recommended to free drinking water from entamœba cysts.

5. That solutions of 4: 1,000,000 calcium hypochlorite ($CaOCl_2$) and 0.5 per cent iodine are also lethal to *E. histolytica* cysts, and may be tried in the treatment of cyst carriers.

ACKNOWLEDGMENTS

The writer is indebted to Dr. Candido M. Africa, head of the Department of Parasitology, School of Hygiene and Public Health, University of the Philippines, for his suggestions and criticisms during the progress of this work, and for his patience in reading the manuscript.

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ILLUSTRATION

[All figures are camera-lucida drawings of *Entamœba histolytica* cysts in the fresh state and after treatment with various dilutions of chlorinated lime solutions. × 1,800.]

PLATE 1

FIGS. 1 and 2. Tetranucleate and binucleate cysts in the fresh state in fæces.
3 to 6. Various stages of degenerated cysts under type I.
7 to 10. Various stages of degenerated cysts under type II.
11 to 14. Various stages of degenerated cysts under type III.
15 to 18. Various degenerated and dead cysts under type IV.

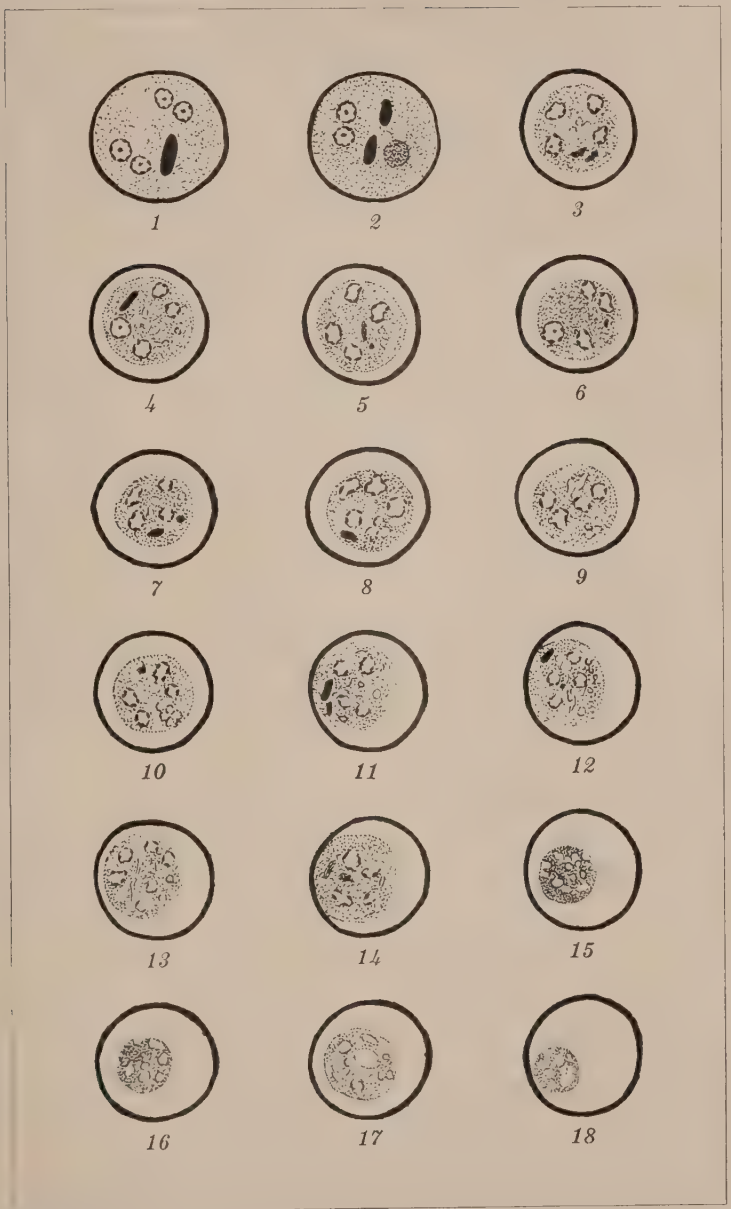

PLATE 1.

STYRAX IN THE PHILIPPINES

By Elmer D. Merrill

Director-in-Chief, New York Botanical Garden

and

Eduardo Quisumbing

Curator, Philippine National Herbarium, Bureau of Science, Manila

ONE PLATE

While the junior author was studying some of the specimens collected in 1930 in the Batan and the Babuyan Islands by Messrs. Gregorio Edaño and Maximo Ramos (deceased), botanical collectors of the Bureau of Science, his attention was called by Mrs. Mary S. Clemens to the possibility of one of the specimens (flowering) being *Styrax*. More critical study of other specimens on hand proved this to be true.

Several botanical expeditions have been made to the Batanes and the Babuyanes, which form the northern portion of the Philippine Archipelago, and a report on a collection of plants from these islands was made by E. D. Merrill.[1] The Batanes and the Babuyanes are separated from Formosa by Bashi Channel. All of these expeditions brought some plants which were new to the Philippines, but known from Formosa. More intensive explorations of these islands will yield further data on the floristic relationships of this group of islands with Formosa.

The family is new to the Philippines except for F. Villar's erroneous record of *Styrax benzoin* Dry. as a Philippine plant, Novis. App. (1880) 127.

STYRACACEÆ

Genus STYRAX Linnæus

STYRAX PHILIPPINENSIS Merr. and Quis. sp. nov.

Frutex 2 ad 3 m altus, ramosus, ramis teretibus, glabris, ramulis gracilibus, pubescentibus. Foliis brevissime petiolatis, ovatis vel ovato-lanceolatis, acuminatis, 4.5 ad 9.5 cm longis, 2 ad 6 cm latis, supra glabris, subtus parce stellato-pubescen-

[1] Philip. Journ. Sci. § C 3 (1908) 385–442, map.

tibus, margine obscure serrulato vel subintegro, nervis utrinque 4 vel 5. Racemis 2- ad 3-floris, breviter pedunculatis, 10 ad 13 mm longis, dense pubescentibus. Calycibus campanulatis, 4 vel 5 mm longis, extus stellato-pubescentibus, intus glabris, 5–lobatis, lobis late triangularibus, 0.5 ad 1 mm longis, apice truncatis vel rotundatis, minute apiculatis. Corolla campanulata; petalis utrinque dense adpresse stellato-hirsutis, oblongo-ellipticis, obtusis, 16 ad 17 mm longis, 7.5 ad 8.5 mm latis. Staminibus 10, aequalibus, 2-seriatis, 9 ad 10 mm longis, filamentis 5 ad 6 mm longis, sursum glabris, deorsum tomentosis. Ovario globoso, tomentoso, stylo 15 ad 20 mm longo, apice truncato. Fructibus ellipsoideis, 12 ad 13.5 mm longis, 8 ad 10 mm diametro, ad basim irregulariter fissis, 1-locellatis, apice rostratis. Seminibus oblongis, 10 ad 11 mm longis, 5 ad 6 mm diametro, 3-sulcatis.

A branching shrub, 2 to 3 m high; stems terete, glabrous, reddish brown or nigrescent, the branchlets slender, pubescent. Leaves very shortly petioled, subchartaceous, ovate to ovate-lanceolate, acuminate, 4.5 to 9.5 cm long, 2 to 6 cm wide, upper surface glabrous, reddish brown or nigrescent, the lower surface paler, stellate-pubescent, margins obscurely serrulate or subentire, the lateral nerves very slender, 4 or 5 on each side of the midrib; petioles 4 to 6 mm long. Racemes 2- or 3-flowered at the ends of the branchlets. Flowers white, pedicels 10 to 13 mm long, densely pubescent. Calyx campanulate, 4 to 5 mm long, sparingly stellate-pubescent without, glabrous within, 5-lobed, the lobes broadly triangular, 0.5 to 1 mm long, apex truncate or rounded, with a minute apiculum. Corolla campanulate; the petals densely adpressed stellate-hirsute on both surfaces, oblong-elliptic, obtuse, 16 to 17 mm long, 7.5 to 8.5 mm wide. Stamens 10, equal, 2-seriate, 9 to 10 mm long; filaments somewhat flattened, 5 to 6 mm long, the upper portion glabrous, the basal part tomentose; anthers linear. Ovary globose, tomentose, 1-celled; style 15 to 18 mm long; stigma truncate. Capsule ellipsoid, apex rostrate, 12 to 13.5 mm long, 8 to 10 mm in diameter. The pericarp ligneous, coriaceous, straw-colored, when dry having a tendency to split irregularly into three valves. Seeds oblong, flat on one side, brownish, 10 to 11 mm long, 5 to 6 mm in diameter, 3-sulcate.

PHILIPPINES, Camiguin Island, Mount Malabsing, *Bur. Sci. 79248 Edaño* (type), March 9, 1930 (flowering specimen).

Batan Island, Mount Matarem, *Bur. Sci. 80424 Ramos*, June 20, 1930 (fruiting specimen).

A species manifestly allied to *Styrax japonicus* Sieb. and Zucc. and to *S. kotoensis* Hayata, of Formosa, and should, we believe, be considered to be a northern type in the Philippine flora. It differs from *S. kotoensis* Hayata, the flowers of which have not been described, in its smaller leaves.

ILLUSTRATION

PLATE 1

Styrax philippinensis Merr. and Quis. sp. nov.: *1*, Habit of flowering branch, × 0.5; *2*, mature flower, × 1.5; *3*, mature flower with two petals and part of calyx removed showing attachment of five stamens and pistil, × 1.5; *4*, petal, flattened, × 2.5; *5*, stamen, × 2.5; *6*, fruit, × 1.5; *7*, seed, × 1.5.

PLATE 1.

LITTLE-KNOWN FISHES FROM THE PHILIPPINES

By Agustin F. Umali

Of the Fish and Game Administration, Bureau of Science, Manila

ONE PLATE

This paper is a systematic description of three rare Philippine fishes based on seven specimens collected from the Manila markets and Lingayen Gulf, Pangasinan Province, mixed with the catches of the beam trawls. They form a part of the ichthyological collection of the Fish and Game Administration, Bureau of Science.

URANOSCOPIDÆ

STARGAZERS

Body elongate, cuboid anteriorly and somewhat compressed posteriorly, widest and usually deepest at occiput. Either naked or covered with very small, cycloid adherent scales arranged in very oblique series running downward and backward; those on belly inconspicuous or obsolete. Head large, flattened above, below, and on sides, almost completely cuirassed with bony plates. Eyes small, on upper anterior portion of head. Mouth vertical with strong and prominent mandible; villiform teeth on jaws, vomer, and palatines; with or without pointed and conical canines; no molars. Maxillary broad, without supplemental bones, not slipping under preorbital. Lateral line continuous, running high. One or two dorsal fins, spinous portion usually much less developed and shorter than soft; anal developed similar to soft dorsal; caudal not forked; pectorals large with broad oblique bases, lower rays rapidly shortened; ventrals jugular, close together, with one spine and five rays. Gill openings wide, continued forward; gill membranes nearly separate, free from isthmus. Branchiostegals 6; air bladder absent.

Carnivorous fishes, living at shore bottoms of tropical seas, northwards to the Mediterranean and Japan, southwards to South Australia and New Zealand.

Genus URANOSCOPUS Linnæus

Uranoscopus LINNÆUS, Syst. Nat. ed. 10 (1758) 250.

Body more or less cylindrical, covered with small and rudimentary scales. Head large, broad, almost wholly covered with bony plates, occipital plates extending to orbits. Opercles and shoulder bones usually armed. Generally a rounded orifice above opercle besides posterior gill opening. Eyes on upper surface of head. Cleft of mouth vertical, generally with a filament below and before tongue. Villiform or cardiform teeth on jaws, vomer, and palatines; no canines. Two separate dorsals, the first with from three to five spines; ventrals jugular. Lateral line continuous, running high. Air vessel absent. Pyloric appendages in moderate numbers.

URANOSCOPUS JAPONICUS Houttuyn. Plate 1, fig. 1.

Uranoscopus japonicus HOUTTUYN, Holl. Maats. Wet. Harlem (1782) 311; JORDAN and SNYDER, Proc. U. S. Nat. Mus. 23 (1901) 745.

Uranoscopus asper TEMMINCK and SCHLEGEL, Fauna Japonica, Poiss. (1842) 26, pl. 9, fig. 1; GÜNTHER, Cat. Fishes 2 (1860) 226; JORDAN and SNYDER, Proc. U. S. Nat. Mus. 23 (1901) 369.

Dorsal III–IV, 14–15; anal 13–14; pectoral 17–18; scales in transverse series about 60.

Body rather elongate, broad anteriorly and compressed toward caudal peduncle; depth 3.6 in length. Head flat above and on sides, with moderately rugose bony plates, dorsal plate extending forward between eyes where it is divided by a deep U-shaped, naked depression; preorbital plate with a flat spine; preopercle with four short spines on lower edge and another stronger one at anterior border of subopercle; humeral spine sharp and long, about 3.2 in length of head,[1] a short flat spine above this. Head 3 in body length; greatest width 1.4 in length of head. Snout very short, 7 in head; mouth vertical; maxillary 2.1 in head; teeth conical and movable in two or three rows in upper and lower jaws, in villiform bands on vomer, palatines, and pharyngeals. Eye small, on upper side of head, 5.8 in head, its horizontal diameter equal to interorbital. Gill rakers on first arch obsolete, represented by a few small bunches of setæ.

Scales small, square, deeply embedded; arranged in about sixty oblique rows; occiput, breast, and belly, as well as a narrow strip along base of dorsal, and a narrow area along base of anal fin

[1] The length of head includes the lower jaw in the description of the species of this family.

naked; lateral line running high, extending along base of dorsal fin abruptly descending to middle of base of caudal at region of caudal peduncle. Dorsals separate, the first with three or four short flexible spines, the highest about 3.5 in head, and the last hardly visible in most specimens as in Plate 1, fig. 1; anterior rays highest, 1.9 in head. Anal inserted below first ray of second dorsal, its fin membrane and especially branched portion of rays fleshy; caudal convex posteriorly, middle rays of pectoral longest; ventrals about 2 in head, three pairs of strong spines directed forward projecting from their bases.

Color of fresh dead specimen, uniform dark brown above, fading to grayish white towards sides, white on belly; spinous dorsal black, basal fin membrane white; soft dorsal and caudal yellowish gray, the former with dusky spots along rays; anal dusky posteriorly, other portions yellowish with white edge; pectorals yellowish with narrow white margin; ventrals white, the edge dusky.

Color in alcohol brownish, fading to whitish toward belly. Spinous dorsal black, with narrow white band at base, the spots along fin rays totally faded; all other fins yellowish, anal and ventrals paler.

Description based on five specimens, No. 31250, total length 150 millimeters, obtained from Quinta Market, Manila, October 25, 1930; Nos. 41063, 41064, 41065, and 51565, 117, 108, 128, and 154 millimeters in total length, respectively; all collected from Lingayen Gulf, Pangasinan, October 3, 1934.

URANOSCOPUS BICINCTUS Temminck and Schlegel. Plate 1, fig. 2.

Uranoscopus bicinctus TEMMINCK and SCHLEGEL, Fauna Japonica, Poiss. (1842) 26, pl. 10, B; BLEEKER, Act. Soc. Nederl., II, Amboina (1857) 411; GÜNTHER, Cat. Fishes 2 (1860) 228; NYSTROM, Svensk. Vet. Akad. (1887) 28; ISHIKAWA, Prel. Cat. (1897) 46.

Dorsal IV, 13; anal 13; pectoral 18; scales in transverse series about 75.

Body robust anteriorly and compressed posteriorly, broader than deep on head; depth 4 in length. Head flat above, covered with coarsely granular bony plates throughout except a narrow U-shaped naked portion at interorbital; preorbital with a flat blunt spine; preopercle with four short spines directed downward on lower edge, another stronger spine at anterior border of suboperculum; humeral spine short and stout, about 5 in head, two short spines above and anterior to this, at superior angle of operculum. Head about 2.7 in body length; greatest width

about 1.2 in length of head. Snout very short, 6.4 in head; mouth vertical; maxillary 2.6 in head; teeth conical and movable, in narrow bands in upper and lower jaws, in villiform bands on vomer, palatines, and pharyngeals. Eye prominent on upper side of head, 7.7 in head, its horizontal diameter contained 1.4 in interorbital. Gill rakers on first arch obsolete, represented by a few small bunches of setæ.

Scales small, square, deeply embedded; arranged in about seventy-five oblique rows; naked at occiput, breast, and belly, as well as a narrow strip along base of dorsal and anal. Lateral line extends along base of dorsal fin, curving downward to middle of caudal fin at region of caudal peduncle. Dorsals separate, first dorsal of four short, flexible spines, the longest 4.5 in head; second dorsal of thirteen rays, third ray longest, 2.2 in head. Anal inserted below origin of second dorsal; fin membrane, especially at branched portion of rays, fleshy; caudal somewhat convex; middle rays of pectoral longest; ventrals 2.1 in head, three strong spines directed forward projecting from base of fin.

Color in alcohol, blackish brown above, fading to grayish white towards lower sides and belly; head and sides of body speckled with minute dark dots; a broad, dark blotch (somewhat indistinct in my specimen) passes around body through base of first dorsal, and an obscure black spot below posterior base of second dorsal. First dorsal black, with a white spot at anterior and posterior of base. Second dorsal dusky, especially towards margins, dotted with black. All other fins dusky yellowish with small black spots; anal, pectorals, and ventrals with white edges.

Here described from a single specimen, No. 31249, total length 262 millimeters, obtained from Divisoria Market, Manila, January 10, 1931.

Found on the coasts of Japan and southward.

OPHIDIIDÆ

CUSK EELS

Body elongate, compressed, more or less eel-shaped; either naked or covered with very small cycloid scales embedded in skin and arranged in oblique series at right angles to each other. Head large, naked or partly scaled; lower jaw included. Mouth moderately protractile, both jaws and generally vomer and palatines also, with villiform or cardiform teeth. Barbels present or absent. Eyes moderate in size, rudimentary or entirely

wanting. Lateral line more or less distinct. Vertical fins of soft rays only; confluent with each other. Pectoral fins sometimes wanting. Ventral fins each reduced to a pair of filaments or a bifid ray inserted just behind chin between rami of lower jaw. Gill openings very wide; gill membranes separate, attached to isthmus behind ventrals. Pseudobranchiæ small; gills 4, a slit behind fourth. Air bladder and pyloric cæca present.

Small, marine carnivorous fishes found in most warm seas, some descending to considerable depths in the Atlantic, Pacific, and Indian Oceans.

Genus LEPOPHIDIUM Gill

Leptophidium Gill, Proc. Acad. Nat. Sci. Phila. (1863) 210.
Lepophidium Gill, Am. Nat. 29 (1895) 167.

Body elongate, more or less compressed, decreasing gradually in height towards posterior where it ends abruptly in a point; scales regularly imbricate in quincunx. Head with imbricated scales extending to forehead; snout high, obtusely rounded, and projecting forward, with or without a short, nearly concealed spine directed forward and somewhat downward; mouth moderate, oblique; teeth in jaws villiform, embedded in a mucous membrane, separated by an interval from longer and pointed ones in outer row; vomer and palatines with teeth.

LEPOPHIDIUM MARMORATUM Goode and Bean. Plate 1, fig. 3.

Leptophidium marmoratum Goode and Bean, Proc. U. S. Nat. Mus. 7 (1885) 423; Goode and Bean, Oceanic Ichthyology (1895) 348, fig. 308.

Dorsal about 90; anal about 60; series of scales along lateral line about 86.

Body more or less elongate and eel-like, moderately compressed anteriorly and ribbonlike posteriorly, and gradually tapering to a point at tail; depth 5.6 in length. Head scaled on cheeks and forehead, 4.5 in body length; snout blunt, without spines, projecting forward over mandible, 4.1 in head, equal to interorbital. Mouth moderate, oblique with villiform teeth on jaws, vomer, and palatines, outer series larger and movable; maxillary extending slightly beyond vertical through posterior margin of orbit, its length equal to postorbital portion of head. Eyes lateral and moderate, 3.2 in head, somewhat exceeding snout. Opercle ending in a small spine somewhat hidden in opercular flap.

Scales small, cycloid, closely imbricate, about eighty-six along lateral line; lateral line apparently complete. Dorsal low, of

about ninety soft rays, confluent with caudal and anal; anal of about sixty soft rays. Pectoral fins short, the length 2 in head. Branchiostegals seven; pseudobranchiæ present; gill-rakers short, eight below angle of first arch, the longest about 0.3 diameter of eye.

Color in alcohol yellowish, lighter towards belly and under-surface of head. A broad brown blotch on forehead continued downward as an oblique band along posterior border of eye to lower edge of opercle; two brown bands on nape before dorsal origin running obliquely downward to belly, the posterior one sending a branch as a longitudinal stripe along whole length of lateral line; another brown stripe along base of dorsal fin. Anterior half of dorsal fin white with irregular blackish to black blotches; posterior half of dorsal, caudal and anal blackish brown, base and outer edges white. Pectorals and ventrals whitish, base of former brownish or dusky.

Description based on a single specimen, No. 31248, total length 140 millimeters, obtained from Divisoria Market, Manila, November 26, 1930.

ILLUSTRATION

[Drawings by Angel D. Lagman.]

PLATE 1

FIG. 1. *Uranoscopus japonicus* Houttuyn.
2. *Uranoscopus bicinctus* Temminck and Schlegel.
3. *Lepophidium marmoratum* Goode and Bean.

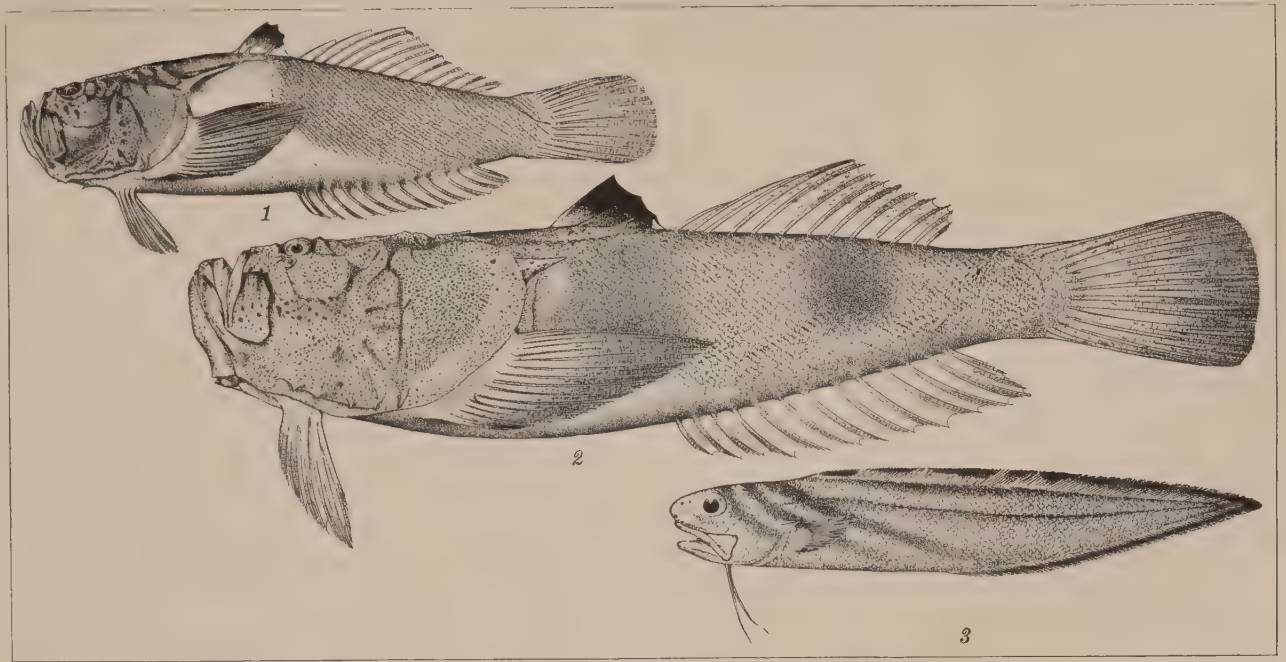

PLATE 1.

A COLLECTION OF SPONGES FROM PUERTO GALERA MINDORO, PHILIPPINE ISLANDS

By M. W. de Laubenfels

Of Altadena, California

ONE PLATE

Prof. Hilario A. Roxas, formerly of the University of the Philippines, has transmitted to me for identification a small but interesting collection of sponges. These were all collected near the marine biological station of the University of the Philippines, which is situated at Puerto Galera Bay, Mindoro Island, Philippine Islands. The sponges are exclusively from very shallow water, in no case more than 12 meters deep. Two of the nine species that could be identified proved to be new, which is very remarkable. It is earnestly hoped that more work will be done with the Porifera at this marine station. Several of the forms in question should prove very interesting for physiological experimentation.

None of them belongs to the class Calcispongiæ, and since this collection is exclusively from shallow waters, none is of the class Hyalospongiæ. The nine species represent nine genera of the class Demospongiæ.

Probably the most extensive treatise on the sponges of the Philippine regions is that by H. V. Wilson.[1]

DYSIDEA PALLESCENS (Schmidt).

The Puerto Galera sponge here described is a massive to irregularly lamellate specimen, gray, tinged with lavender, as preserved in alcohol. Its consistency is spongy. There is a smooth, presumably horny, dermis, as is true of keratose sponges in general, and it is stretched over conules 2 mm high and 1 to 8 mm apart. Underneath this dermal membrane there probably have been subdermal cavities, but in general these are collapsed at the time of examination. The pores and oscules are not evident. In the endosome there is an obvious reticulation of the fibers. The flagellate chambers are eurypyllous, 40 μ

[1] Bull. U. S. Nat. Mus. 100 (1925).

by 65 μ, oval. Of the above-mentioned fibers, the mesh, approximately square, is about 0.45 to 1 mm in diameter. The fibers are about 360 in diameter and are crowded with foreign material so that the spongin is scarcely evident. The fascicular nature of the specimen studied by Wilson is barely in evidence, but there can be little doubt that it is the same variety as the one found by that author near this locality.

This specimen is in general quite characteristic of *pallescens*, especially in the lavender color and the wide distribution of the conules. The other conspicuous member of the genus *Dysidea* is *D. fragilis*, which is a pale gray sponge lacking the lavender and having many small conules very close together, which are usually less than 1 mm high and less than 1 mm apart, apex to apex.

Remarks.—This species was described as *Spongelia pallescens* by Schmidt (1862, p. 29) in his monograph on the sponges of the Adriatic, and referred to *Spongelia* by Schulze (1879, p. 138). There may be some doubt that the Philippine sponge is conspecific with Schmidt's from the Mediterranean. Wilson (1925, p. 476) reported from the Philippine Islands a sponge which is clearly conspecific with that in the collection at present under discussion, and identified it as *Dysidea fragilis* var. *fasciculata* Wilson. Wilson explained that he employed the specific name in question rather than *pallescens* because Lendenfeld (1889, p. 642) had reduced *pallescens* in synonymy to *Spongia fragilis* of Montagu (1818, p. 114). But this is not a good reason. Hallmann [2] demonstrated conclusively that the latter author was utterly unreliable in his conclusions and descriptions.

ADOCIA BAERI (Wilson). Plate 1, fig. 1.

The Puerto Galera specimen is represented by some fragments, perhaps all from the same colony, which colony was presumably ramose. The fragments are cylindrical, usually hollow, and in one case dichotomously branched. The color as preserved in alcohol is dark, almost black. The consistency is very soft. The surface, as is very characteristic of *baeri*, is minutely tuberculate. A dermal region of specialized pigment cells extends some 400 μ into the sponge. The endosome is a typical "renierid" or isodictyal reticulation, with conspicuous canals about 1 mm in diameter, running longitudinally in the cylinders, parallel to the surfaces of the exterior and of the cloacal hollow.

[2] A Revision of the Monaxonid Species Described as New in Lendenfeld's Catalogue of the Sponges in the Australian Museum (1914).

There are what appear to be pores, remarkably large, even 1 mm in diameter in many cases. The oscule or cloacal aperture is terminal and 3 mm in diameter. The megascleres are rather sharp pointed oxeas, fairly uniform in size, 5 μ by 130 μ. Among them are considerable numbers of much smaller oxeas, only 0.5 μ by 90 μ. These are perhaps undeveloped megascleres of the principal type or, on the other hand, perhaps to be regarded as microscleres. Spicules, while not yet fully formed, are well known to resemble the fully formed ones in shape, except for much greater slenderness, and the presence of such spicules must therefore have very doubtful taxonomic value. It is always possible that they may represent a special category, but it can seldom be ascertained to any degree of satisfaction whether or not this is the case.

Remarks.—Wilson (1925, p. 398) reported from the Philippine Islands a specimen obviously conspecific with the present variety, which he identified as *Reniera implexa* Schmidt var. *baeri* Wilson (1868, p. 27). Burton[3] demonstrates the suitability of the genus *Adocia* rather than *Reniera* for cases such as the one under consideration; furthermore, the differences between the Philippine and the Mediterranean forms are sufficiently great for full specific rank. Within the genus under discussion there are so few criteria upon which to base specific differences that items which would appear trivial in other groups must here receive major attention.

GELLIODES FIBROSA (Wilson). Plate 1, fig. 2.

The Puerto Galera species here described is a small cavernous mass represented in places by only a series of trabeculæ each about 2 by 4 mm. As preserved in alcohol it is blue-gray on the exterior over a paler gray not at all blue on the interior. The consistency is quite spongy. The ectosomal specialization is a membranous sheet, transparent, and contains tangentially placed spicules. The endosome is very cavernous, with chambers 6 mm in diameter, elongate, meandering through the interior. The architecture is pronouncedly fibrous as is characteristic of the genus *Gelliodes* but not of the so-called *Gellius*. These fibers contain many spicule rows, and range from 60 μ to 160 μ in diameter. The mesh that they form is commonly 700 μ to 2,000 μ in diameter. There are auxiliary connectives which are fibers of only about half (or less) the diameter of the

[3] Sponges of the Great Australian Reef (1934).

principal ones, and these inclose meshes only 40 μ to 60 μ in diameter. The spicules are typically oxeas, but perhaps 3 to 5 per cent of them are styles. Typical sizes are 6 μ by 190 μ, 5 μ by 175 μ, 5 μ by 150 μ, 4 μ by 150 μ. Among these are sigmas of great variation in size and thickness; the length of chord varies from 11 μ to 22 μ, and the thickness from much less than to nearly 2 μ.

The cavernous structure is perhaps the most characteristic feature of this species as contrasted with others of the genus *Gelliodes*. The spicules are rather smaller than those of most, and the lack of conules also is noteworthy.

Remarks.—This sponge was described from the Philippines by Wilson (1925, p. 398) as being conspecific with the sponge first described as *Halichondria varia* by Bowerbank (1875, p. 292), and later referred to *Gellius* by Ridley and by other authors. Wilson established for the Philippine sponge a new variety, *fibrosa*. The present specimen sheds still further light on the nature of the species over and above the information available to Wilson, and it now becomes evident that instead of being a *Gellius* (*Adocia*), this is a *Gelliodes*. Therefore, it becomes necessary to treat the name *fibrosa* as of full specific rank instead of as a variety. It is not implied that *varia* of Bowerbank is also a *Gelliodes*, but rather that it is not even congeneric with the Philippine sponge.

LISSODENDORYX ROXASI sp. nov. Plate 1, fig. 3.

This Puerto Galera sponge is about 2 cm by 6 cm, an irregular reticulation of trabeculæ, very cavernous in nature. The color is drab, and the consistency is very elastic. Because of the cavernous, perhaps partially macerated condition, the pores and oscules cannot be described. A large amount of the dermis may have been lost. The reticulation made by the structures referred to above consists of meshes only some 2 mm apart, center to center, and nearly rectangular in outline. The trabeculæ themselves are nearly 1 mm in diameter. The ectosomal spicules are uncommon, many having perhaps been lost since the sponge was preserved. They are tylotes, 3 μ by 220 μ. The abundant principal spicules are crowded rather irregularly in the endosome. They are entirely spined styles, 8 μ by 155 μ. Many of them are so placed as to echinate the trabeculæ. There are two size ranges of arcuate isochelas. The abundant ones are about 36 μ long, but a rarer sort is only 16 μ long. Sigmas 70 μ in chord length are present, but not at all common.

Remarks.—Wilson (1925, p. 432) described a species of *Lissodendoryx,* from the Philippine Islands, naming it *tawiensis.* There is little reason to believe that this species is conspecific with the form under consideration. In fact, it is not even congeneric, especially since its chelas are not arcuate, but distinctly palmate. It is, therefore, here proposed to regard it as the genotype of a new genus to be known as **Myxichela.** The diagnosis would be, "resembling *Myxilla* except for the substitution of palmate chelas instead of any other kind." The nearest of the numerous species of *Lissodendoryx* to *roxasi* seems to be that described by Topsent (1897, p. 457) as *baculata,* from the East Indies. Of the latter, the dermal spicules are strongyles instead of tylotes, but of about the same size as those in *roxasi.* The principal megascleres are somewhat larger, and smooth. It has only the smaller size range of isochelas, and its more numerous sigmas are smaller.

The genus *Lissodendoryx* is very close to the genus *Myxilla* from which it was originally separated by having smooth principal spicules, whereas *Myxilla* has spiny megascleres. This fact by itself alone proved to be an untenable basis of differentiation, and it was noted that the genotype has this further significant difference, that its chelas are arcuate instead of anchorate, as in *Myxilla,* and this distinction has been recently accepted as the firm basis for differentiation. However, there are very few species of *Lissodendoryx* that possess spines upon the principal megascleres; the sponge now being described is one of the few presenting this characteristic.

The species described here is named in honor of Prof. Hilario A. Roxas, of the Bureau of Science, Manila.

MONANCHORA DIANCHORA sp. nov. Plate 1, fig. 4.

This Puerto Galera sponge was a lamellate mass about 1 cm thick, and 4 by 5 cm in area, curled in such a way as almost to make a funnel. Preserved in alcohol, it is light orange-red on the exterior and paler orange on the interior. The consistency is soft and spongy. The surface might perhaps be described as conulose, but there are only about a dozen conules on the entire surface, which is otherwise smooth. The conules in question are about 4 mm high. The dermal membrane is thin and can only with great difficulty be detached from the subdermal cavities which extend beneath it. In it the dermal spicules are tangentially placed. The endosome is very confused, but vague ascending tracts can be made out here and there,

consisting of approximately eight spicule rows each, and being about 40 μ in diameter. The megascleres are of one type only, namely tylostyles, but tend to be of two sizes. Those in the ectosome are approximately 4 μ by 283 μ. Typical sizes for those in the endosome may be given as 7 μ by 276 μ, 5 μ by 370 μ, 9 μ by 270 μ. The microscleres are most distinctive. There are two kinds, appearing superficially like sigmas, but being really unguiferate chelas having several teeth at each end. The commonest category is of isochelas of this type, 33 μ in chord length, and having at each end four sharp-pointed teeth. A second category is of similar spicules, only 20 μ in chord length and apparently having only three minute teeth at each end.

Remarks.—This sponge may be compared with *Monanchora clathrata* Carter (1883, p. 369), the type of the genus, which it resembles in general, except that Carter's species had at least five teeth at each end of the peculiar chelas. We do not have many data pertaining to the Australian species, there being little information available, for example, as to its flesh and structure in general. Another species of especial interest here is the one described by Dendy (1921, p. 58) as *Amphilectus unguiculatus*, from the Indian Ocean. Its megascleres are considerably longer but not thicker than those of *dianchora*, and its microscleres are very unusual in their flat, straplike shape, the shaft not being round in cross section as might be expected.

It is interesting to find another representative of this rare genus characteristic of the East Indian and Australian regions. It is somewhat paradoxical that Carter's choice of a generic name was most inappropriate. Whereas the Philippine species has more than one kind of "anchor," it is clearly so closely related to *clathrata* that it scarcely seems appropriate to establish for it at the present time a new genus, merely because it does not conform to the description implied in the generic name.

DRAGMAXIA CILIATA (Wilson). Plate 1, fig. 5.

The Puerto Galera sponge here described is a lobate mass about 7 by 3.5 by 3.5 cm. The surface is smoothly rounded into somewhat less than a dozen nearly spherical lobes. The color as preserved in alcohol is brown, darker on the exterior, and paler in the interior. The consistency, like that of the genus *Suberites*, is corklike. The surface, however, is pronouncedly hispid, with a spicule fur about 80 μ high consisting of spicules some 20 μ apart, placed erect; otherwise the surface

may be described as nearly smooth. There are numerous pores 28 μ to 40 μ in diameter, and about 80 μ to 90 μ apart, but the oscules cannot be made out with certainty. The endosome is pronouncedly radial, and very dense in structure, lacking the breadlike appearance frequently found in sponges of the Suberitidæ. The flagellate chambers are round, presumably diplodal, varying from 28 μ to 40 μ in diameter. The megascleres are styles. Those of the ectosome are only about 4 μ by 320 μ, a few being as much as 5 μ in diameter, but many only as thick as 2 μ. Those of the endosome are much larger, varying frequently between 22 μ by 930 μ and 26 μ by 1040 μ. Among them are very small spicules or rhaphides less than 1 μ in diameter and usually a little more than 200 μ in length.

Remarks.—Wilson (1925, p. 341) described this species from the Philippines as *Tuberella ciliata.* Topsent in 1900 in his "Etude monographique des Spongiaires de France" made clear that *Aaptos* and *Tuberella* were synonymous. The former dates from Gray (1867) and the latter only from Keller (1881), so it is evident that *Tuberella* should be dropped in favor of *Aaptos,* not vice versa. It appears that Wilson's specimen had few or none of the rhaphides, so *Dragmaxia* was not suggested.

The genus *Dragmaxia* has for a long time been represented exclusively by its genotype, the sponge described by Whitelegge (1907, p. 513) as *Spongosorites variabilis.* It is quite a pleasure to find another species for this rare and interesting genus. Whitelegge established the genotype with the understanding that his species was an epipolasid, that is to say, a reduced tetraxon sponge. Apparently Hallmann (1916, p. 543) was quite right when he transferred it (as of his new genus *Dragmaxia*) to the Axinellidæ. A still further interesting concept is brought out by comparison with the genus *Aaptos; Dragmaxia ciliata* is very much like *Aaptos,* except for its possession of oxeote microscleres. The latter genus is usually regarded as suberitid rather than axinellid. The dense subspherical shape of *ciliata* suggests a suberitid relationship, but its hispid ectosome points more forcibly toward the Axinellidæ. All items considered, this is not only an interesting but also a perplexing species.

Comparing this sponge with the other species of *Dragmaxia* mentioned above, that species (*variabilis*) is lamellate, and its principal spicules are nearly double the thickness without

being longer than those of *ciliata;* its microscleres are about five times as thick without being longer. A fairly close relationship is indicated. Another genus that is worthy of consideration here is *Alloscleria* Topsent, which, however, has two types of diactinal microscleres, one kind acanthoxeas, and the other centrotylote smooth oxeas.

SPIRASTRELIA VAGABUNDA Ridley. Plate 1, fig. 6.

The present Puerto Galera species is a compact mass 4 cm in diameter and 6 cm in height, showing pronounced vertical grooves on the lateral surfaces, so that it becomes almost digitate. In alcohol it is a rich brown, and the consistency resembles that of cork. The color is due to very strongly pigmented bodies contained in a layer of cells about 40 μ deep from the surface. In general the surface is to be described as smooth. No membrane can easily be removed from it. The structures in the interior are as confused as are those of the exterior, but more of the spicules have their points directed towards the surface of the sponge than in any other direction. There are no special dermal spicules. The megascleres are of one type only, namely tylostyles, of which the following sizes are representative: 13 μ by 570 μ, 14 μ by 540 μ, 16 μ by 594 μ, 14 μ by 634 μ, 9 μ by 405 μ. The microscleres are much bent or spiral spirasters with very small spines; the entire spicule averages little more than 1 μ in thickness, and the maximum length is about 15 μ.

Remarks.—Very numerous species of *Spirastrella* have been described from various parts of the world and they do not differ greatly from each other. Vosmaer in 1911 in connection with the *Siboga* reports, published a monograph on this genus, in which he reduced a very large number of species in synonymy to *Spirastrella purpurea.* It is here considered that Wilson's remarks in the 1925 reference mentioned above are correct; namely, that by no means all of the species mentioned by Vosmaer should be so reduced.

The sponge at present under consideration is obviously the same as that described by Wilson (1925, p. 343) with the above identification. This statement calls for some discussion, but little more need be said than what has already been written by Wilson in the reference cited. *Vagabunda* was established by Ridley (1884, p. 468), in his report on the Porifera collected by the *Sea Lark* in the Indian Ocean.

MYRIASTRA CLAVOSA (Ridley). Plate 1, fig. 7.

This Puerto Galera sponge is represented by a number of small spherical masses some 6 to 8 mm in diameter each. The color as preserved in alcohol is very pale brown, almost white, and the consistency is rather elastic but similar to cork. The cortical specialization is very thin for a tetraxon sponge of this nature, being only from 80 μ to 95 μ thick. It extends over very definite subdermal cavities. The endosomal structure is pronouncedly radial. Pores cannot be distinguished with certainty, and each of the small sponges seems to have just one oscule, which is about 1 mm in diameter. The principal spicules are large dichotriænes with their cladomes in or just below the cortex. They are frequently as long as 4 mm. The rhabds are about 50 mm in diameter, and the chord ranges from 570 μ to 660 μ. There are anatriænes, protriænes, and orthotriænes, as well as oxeas. The rhabds of the former, and the total diameter of the latter, are in the neighborhood of 18 μ. The chords of the triænes range from about 65 μ to nearly 100 μ. Microscleres seem to be of one sort only, namely tylasters. They vary from 9 μ to 15 μ in diameter, and (as usual among the tetraxon sponges) the smaller asters have the more numerous rays, up to fourteen or fifteen, whereas the larger have less numerous rays, only three to five.

Remarks.—This species was first described as *Steletta clavosa* by Ridley (1884, p. 474), in his report of the Indian Ocean sponges collected by the *Sea Lark*. A very complete and satisfactory discussion of it is given by Wilson (1925, p. 387).

GEODIA SPARSA Wilson.

The Puerto Galera sponge here described is a subspherical mass about 1 cm in diameter, light brown in color, and with a stony hard cortex. Underneath this the endosome is brittle but somewhat like cork in other ways. These characters are quite typical of the genus *Geodia*. The cortex is about 800 μ thick, and is densely packed with sterrasters as characteristic of the group. The pores and oscules cannot be distinguished. The endosome is pronouncedly radial in structure. The principal megascleres are plagiotriæna with rhabds about 20 μ by 1685 μ (a few being much longer), and with chords 265 μ across. In this specimen there occur also orthotriænes of which the rhabds appear always to have been broken. The clads are about 350 μ to 430 μ long. The sterrasters are approxi-

mately 108 μ by 121 μ, and, of course, developmental forms thereof occur. The euasters appear to have their arms microspined; they reach a diameter of approximately 12 μ to 16 μ. There are furthermore rather numerous spherasters, chiefly centrum, having a diameter of barely 4 μ.

Remarks.—This sponge was described as a new species from the Philippines by Wilson (1925, p. 314).

ILLUSTRATION

PLATE 1

[From camera-lucida drawings. Figs. 1 to 4 and 7, *e* are × 630; figs. 5, 6, and 7, *a* to *d* are × 150.]

FIG. 1. *Adocia baeri* (Wilson); *a*, oxeote megasclere; *b*, rhaphide.
2. *Gelliodes fibrosa* (Wilson); *a* and *b*, oxeas; *c*, sigmas.
3. *Lissodendoryx roxasi* sp. nov.; *a*, tylote; *b*, acanthostyle; *c*, sigma; *d*, larger (arcuate) chelas; *e*, smaller chelas.
4. *Monanchora dianchora* sp. nov.; *a*, tylostyle; *b*, larger (unguiferate) chelas; *c*, smaller chelas.
5. *Dragmaxia ciliata* (Wilson); *a*, endosomal style; *b*, ectosomal style; *c*, rhaphide.
6. *Spirastrella vagabunda* Ridley; *a*, tylostyle; *b*, spirasters.
7. *Myriastra clavosa* (Ridley); *a*, cladome of dichotriæne; *b*, cladome of plagiotriæne; *c*, protriæne; *d*, cladome of anatriæne; *e*, tylasters.

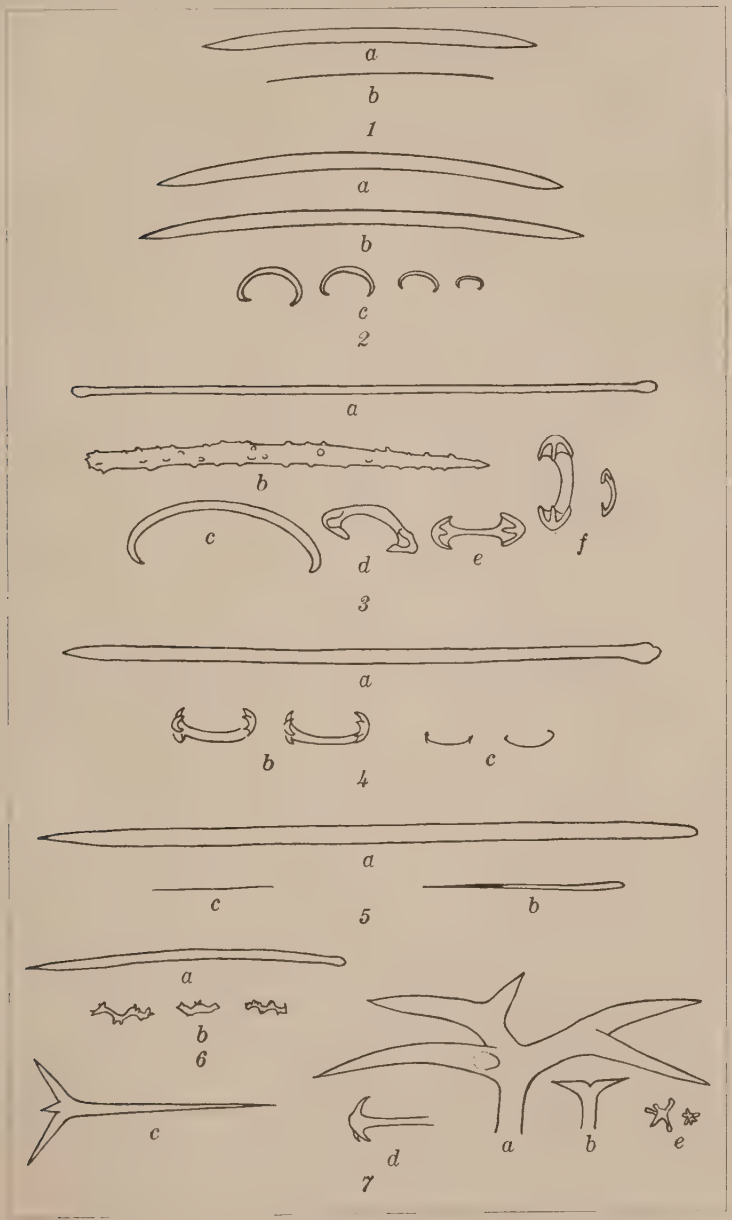

PLATE 1.

NEW OR LITTLE-KNOWN TIPULIDÆ FROM EASTERN ASIA (DIPTERA), XXIII[1]

By CHARLES P. ALEXANDER

Of Amherst, Massachusetts

THREE PLATES

The crane flies discussed in this paper are chiefly derived from three sources; namely, Celebes, collected by Mr. Charles F. Clagg; Formosa, by Mr. J. Linsley Gressitt; and Kashmir, by Miss Vivien R. Hutchinson. Scattered additional species were secured in Java by Dr. Ian Mackerras; in Formosa by Prof. Syûti Issiki; and on Mount Omei, western China, by the Rev. Mr. George M. Franck. One further species, from eastern Siberia, was included in the rich collections belonging to the Russian Academy of Sciences, Leningrad, sent to me for study by Dr. A. von Stackelberg. I wish to express my deepest thanks to all of the above-named entomologists for their continued kindly coöperation in my study of the Tipulidæ of Asia. Except where indicated to the contrary, types of the various novelties are preserved in my collection. As has been done in other parts under this series of reports, I am including a species of Trichoceridæ, preferring this course to preparing a special paper on this insect.

TRICHOCERIDÆ

TRICHOCERA ARISANENSIS sp. nov. Plate 1, fig. 1.

General coloration of thorax brownish yellow, the præscutum with three darker brown stripes; pleura chiefly dark brown, variegated by paler brown; femora brown, their bases obscure yellow; wings yellowish gray, sparsely patterned with brown; abdominal segments conspicuously bicolorous, the basal portion brown, the outer ring light yellow.

Female.—Length, about 6 millimeters; wing, 6.5.

Rostrum dark brown; palpi elongate, brownish black. Antennæ black, the scape and pedicel paler; outer flagellar segments

[1] Contribution from the entomological laboratory, Massachusetts State College.

paler than the basal ones; flagellum much more elongate and much slenderer than in *pictipennis*, the individual segments correspondingly lengthened. Head brown.

Mesonotal præscutum brownish yellow with three darker brown stripes; scutal lobes dark brown, the median area more yellowish; scutellum and mediotergite more testaceous brown. Pleura chiefly dark brown, variegated by paler brown on the pteropleurite and posterior anepisternum; dorsopleural membrane yellow. Halteres pale yellow, the knobs dark brown. Legs with the coxæ and trochanters yellowish testaceous; femora obscure yellow basally, passing into brown outwardly; tibiæ and tarsi brown. Wings (Plate 1, fig. 1) with the ground color yellowish gray, sparsely patterned with brown, including areas at origin of Rs, along cord, on vein R_2, outer end of cell 1st M_2, and the fork of vein M_{1+2}; paler and less evident areas in the outer radial field; no darkening at end of vein 2d A; veins brown. Venation: R_{2+3+4} a little shorter than R_{1+2}; Sc_1 ending about opposite fork of R_{2+3+4}; cell M_1 subequal to or a little longer than its petiole; m-cu shortly before fork of M_{3+4}; vein 2d A somewhat angular, at extreme outer end bent suddenly into margin.

Abdominal segments conspicuously bicolorous, the basal two-thirds of the individual segments brown, the outer third light yellow. Cerci pale yellow, the tips acute.

Habitat.—Formosa.

Holotype, female, Arisan, altitude 7,640 feet, May 24, 1934 (*Gressitt*).

The nearest allied species is *Trichocera pictipennis* Alexander, of Japan and Korea, which differs conspicuously in the coloration of the thorax and abdomen, in the shorter antennæ, and in the details of the wing pattern. The genus *Trichocera* is new to the fauna of Formosa.

TIPULIDÆ

TIPULINÆ

LONGURIO (LONGURIO) VARICEPS sp. nov. Plate 1, fig. 2.

General coloration of thorax black; head fulvous-orange, with a conspicuous black longitudinal stripe on either side of vertex; halteres and legs black throughout; wings strongly tinged with black, the costal portion and seams along vein Cu and anterior cord still darker; Rs short, a little less than cell 1st M_2; abdomen with segments six to nine, inclusive, black; tergites two to five

orange, narrowly bordered by blackish; basal sternites conspicuously bicolorous, black, variegated by yellow.

Male.—Length, about 21 millimeters; wing, 16.5; abdomen alone, 16.5.

Female.—Length, about 25 millimeters; wing, 18; abdomen alone, 20.

Rostrum and palpi velvety black. Antennæ black throughout; outer verticils very long and conspicuous. Head dull fulvous-orange, with a conspicuous black longitudinal stripe extending from either side of the vertical tubercle along the orbital region, behind becoming more diffuse and involving the occipital region, leaving the median area of the posterior vertex of the ground color.

Thoracic dorsum and pleura entirely deep velvety black. Halteres and legs black throughout. Wings (Plate 1, fig. 2) very strongly tinged with black, the prearcular region, cells C and Sc, stigma, and seams along vein Cu and on anterior cord darker; paler longitudinal streaks in cells R, M, and 1st A in female sex; veins black. Venation: Rs shorter than cell 1st M_2 and a little longer than m-cu.

Abdomen with basal segment velvety black; tergites two to five, inclusive, orange, narrowly bordered on the lateral and caudal portions by black; succeeding tergites and hypopygium black; basal sternites conspicuously bicolorous, black, variegated more extensively on sides of basal ring with yellow, less extensively and distinctly on outer ring just before apex.

Habitat.—Formosa.

Holotype, male, Hori, altitude 2,340 feet, June 7, 1934 (*Gressitt*).

Allotopotype, female. Paratopotype, female.

The nearest ally is *Longurio* (*Longurio*) *rubriceps* Edwards, likewise from Formosa, which differs in the coloration of the head, legs, and pattern of the basal abdominal sternites, and in the elongate Rs.

TIPULA (ACUTIPULA) ALBOPLAGIATA sp. nov. Plate 1, fig. 3; Plate 2, figs. 25, 26.

Allied to *Tipula* (*Acutipula*) *munda;* antennal flagellum weakly bicolorous, the basal enlargements of the individual segments a trifle darker than the remainder; pleura yellow; legs black; wings very strongly tinged with brown, variegated by a whitish obliterative area before cord and by a pale yellow longitudinal streak behind vein Cu; abdomen yellow, the outer segments brownish black; male hypopygium with tergal lobe

bifid; inner dististyle with outer lobe terminating in a very slender, acute spine; eighth sternite fringed with yellow setæ.

Male.—Length, about 17 millimeters; wing, 19.5.

Frontal prolongation of head brown; nasus dark brown, conspicuous; palpi black. Antennæ with scape and pedicel dark brown; flagellum very weakly bicolorous, the basal enlargement a trifle darker brown than the remainder. Head dark brownish gray.

Pronotum uniformly reddish brown. Mesonotal præscutum gray, with four more olive-gray stripes that are poorly defined against the ground, the stripes feebly delimited by very narrow darker borders; scutal lobes brownish gray, the median region more yellowish; posterior sclerites of mesonotum chiefly yellow pollinose. Pleura yellow, the pleurotergite gray pruinose. Halteres brownish black throughout. Legs with the coxæ and trochanters yellow; remainder of legs black, excepting only the very narrow femoral bases which are yellow. Wings (Plate 1, fig. 3) very strongly tinged with brown, the prearcular region and cells C and Sc even darker brown; stigma small, yellow; ground color variegated by a conspicuous whitish obliterative area before the cord, extending from the proximal end of the stigma into cell 1st M_2 but not or scarcely invading cell M_3; a conspicuous, pale yellow, longitudinal stripe extending almost the entire length of wing immediately behind the basal section of vein Cu_1, involving cell Cu_1 and the adjoining portion of cell Cu; veins dark brown. Macrotrichia of veins sparse; squama with setæ. Venation: Petiole or cell M_1 shorter than m.

Abdomen yellow, the tergites with a narrow, brown, sublateral border; segments six to nine, inclusive, more uniformly brownish black. Male hypopygium (Plate 2, fig. 25) with the ninth tergite (Plate 2, fig. 26, 9*t*) narrowed at outer end into a depressed yellow lobe, the apex of which has a deep U-shaped median notch, the slender lobes thus formed set with blackened spines. Outer dististyle, *od*, flattened, relatively narrow, the outer margin nearly straight, the inner one evenly curved. Inner dististyle, *id*, with the outer lobe terminating in a very slender, acute spine; inner lobe darkened, simple, the apex obtuse. Eighth sternite (Plate 2, fig. 26, 8*s*) with the caudal margin transverse, fringed with yellow setæ, those at the outer lateral portions a little longer and slightly stouter.

Habitat.—Formosa.

Holotype, male, Arisan, altitude 7,640 feet, May 24, 1934 (*Gressitt*).

Tipula (*Acutipula*) *alboplagiata* is very different from other regional members of the *munda* group in the black legs, darkened wings, and structure of the male hypopygium, especially of the inner dististyle which is distinct from that of all other described species known to me.

TIPULA (SCHUMMELIA) INDIFFERENS sp. nov. Plate 1, fig. 4; Plate 2, figs. 27, 28.

Belongs to the *continuata* group; antennæ with flagellar segments unicolorous; mesonotal præscutum obscure brownish yellow, with three brownish gray stripes, the median one divided by a capillary blackish line; knobs of halteres chiefly light yellow; wings grayish brown, the stigma dark brown; restricted paler brown clouds in outer end of cell 1st M_2, on m-cu, and on distal section of Cu_1; squama naked; abdomen orange, segments six to nine uniformly black.

Male.—Length, 12 to 13 millimeters; wing, 13 to 14.5.

Frontal prolongation of head dark brown above, paling to yellow beneath and on sides; nasus elongate. Antennæ (male) of moderate length, if bent backward extending about to mid-distance between the bases of the wings and halteres; scape and pedicel yellow; basal two or three flagellar segments brownish yellow, the succeeding segments passing into black; verticils a little longer than the segments. Head brownish gray, more or less pruinose.

Mesonotal præscutum obscure brownish yellow, with three brownish gray stripes, the median one divided by a capillary more-blackish line; lateral stripes vaguely bordered by darker, especially along their mesal edge; centers of scutal lobes darkened; scutellum infuscated basally, margined with yellow; postnotum yellow. Pleura chiefly yellow or brownish yellow, the pleurotergite darker and weakly pruinose. Halteres dusky, the base of stem obscure yellow, the knob chiefly light yellow. Legs with the coxæ and trochanters yellow; femora obscure yellow, before the tips narrowly blackened, the extreme apices restrictedly pale; tibiæ brown, the tips slightly darker; tarsi black. Wings (Plate 1, fig. 4) with the ground color grayish brown, very weakly patterned with darker; stigma oval, dark brown; paler brown clouds across outer end of cell 1st M_2, along m-cu and outer section of vein Cu_1; obliterative area extensive, from the outer end of cell R into base of cell M_3; veins dark brown. Squama naked. Venation: R_{1+2} entire but without trichia except at base; Rs shorter than m-cu; petiole of cell M_1 shorter than m.

Abdomen orange, the pleural region narrowly dusky; segments six to nine uniformly black, only the outer dististyle pale yellow. Male hypopygium (Plate 2, fig. 27) with the caudal border of the tergite (Plate 2, fig. 28, 9*t*) deeply emarginate, blackened, fringed with delicate yellow setæ. Outer dististyle, *od*, slightly flattened, narrowed outwardly, the tip obtuse. Inner dististyle as shown (Plate 2, fig. 28, *id*); setæ of outer margin approximately 20 in number.

Habitat.—Kashmir.

Holotype, male, Srinagar, altitude 5,000 feet, June 9, 1934 (*Miss Hutchinson*). Paratopotype, male; paratypes, 3 males, Kaj-Nag Range, altitude 8,000 feet, May 30 to June 1, 1934.

Tipula (*Schummelia*) *indifferens* is very distinct from the other regional members of the subgenus in the almost unpatterned wings and the conspicuous coloration of the abdomen. The nearest allies would seem to be *continuata* Brunetti and *xanthopleura* Edwards, both with a very distinct pattern of the wings and abdomen.

TIPULA (SCHUMMELIA) NIGROCELLULA sp. nov. Plate 1, fig. 5; Plate 2, fig. 29.

Belongs to the *continuata* group; præscutal stripes distinct, narrowly bordered by darker brown; thoracic pleura striped longitudinally with brown; knobs of halteres extensively yellow; femora with a narrow, nearly terminal, black ring; wings whitish subhyaline, with a conspicuous brown pattern; cell Sc uniformly blackened; cell 2d A yellow, variegated by two dark clouds; squama naked; abdominal tergites reddish, narrowly lined with darker; a subterminal black ring on abdomen; male hypopygium with the caudal margin of tergite tridentate; basistyles bearing conspicuous pencils of reddish setæ.

Male.—Length, 12 to 13 millimeters; wing, 12.5.

Female.—Length, 17 to 19 millimeters; wing, 15 to 16.

Frontal prolongation of head yellow above and beneath, narrowly darkened on sides; nasus elongate, yellow. Antennæ (male) of moderate length, if bent backward extending approximately to wing root; scape and pedicel light yellow; flagellar segments dark brown, the extreme outer end of the individual segments a trifle paler; longest verticils subequal to the segments. Head brownish yellow, clearer yellow on anterior vertex; a narrow brownish median line.

Mesonotal præscutum yellow, with three conspicuous brown stripes that are narrowly bordered by darker brown, the median one further divided by a dark vitta; scutal lobes chiefly covered

by brown areas, the median region of scutum pale; scutellum and mediotergite chiefly darkened medially, paler laterally. Pleura yellow, conspicuously striped longitudinally with dark, including a dorsal stripe extending from the propleura across the ventral anepisternum, and a more interrupted, fainter area on the ventral sternopleurite and meron. Halteres with the stem obscure yellow, a little brighter at base; base of knob brown, the apex conspicuously light yellow. Legs with the coxæ and trochanters pale yellow; femora brownish yellow, immediately before tip with a narrow black ring; tibiæ brown, paler basally, blackened outwardly; tarsi black. Wings (Plate 1, fig. 5) whitish to pale yellow subhyaline, handsomely patterned with brown; prearcular region and cell C clearer yellow; cell Sc uniformly blackened, excepting the yellow extreme outer end; stigma dark; the dark discal pattern includes conspicuous clouds across m-cu and fork of Cu; at near midlength of cell M, and at outer end of cell 1st M_2, together with the adjoining veins; outer radial cells weakly washed with brown; cell 2d A yellow, conspicuously variegated by a dark cloud at end and by another at midlength of margin; veins dark, except in the obliterative area. Squama naked. Venation: R_{1+2} entire; petiole of cell M_1 subequal to or shorter than m.

Abdominal tergites chiefly reddish, the sternites more yellow; tergites narrowly and inconspicuously lined medially and laterally with black; a black subterminal ring, involving tergites six to nine and sternites seven and eight; remainder of the hypopygium yellow. Male hypopygium with the tergite (Plate 2, fig. 29, 9*t*) transverse, the emarginate caudal end tridentate, with smaller blackened teeth on either side, directed mesad, together with a paler median tooth. Region of basistyle, on either side, with a pencil of reddish setæ, directed ventrad. Outer dististyle relatively long and slender, subterete, narrowed gradually to the apex. Ninth sternite on either side of ventral portion, immediately caudad of the margin of the eighth sternite, produced ventrad into a reddish fingerlike lobe.

Habitat.—Kashmir.

Holotype, male, Kaj-Nag Range, altitude 8,000 feet, June 1, 1934 (*Miss Hutchinson*). Allotopotype, female. Paratopotypes, 1 male, May 25, 1934; 1 male, 1 female, altitude 9,000 feet, May 30 to June 3, 1934.

The present species is readily told from all other regional members of the subgenus by the uniformly blackened subcostal cell and by the rather peculiar structure of the male hypopygium.

TIPULA (SCHUMMELIA) INDISCRETA sp. nov.

Female.—Length, about 17 millimeters; wing, 15.5.

Generally similar to *T.* (*S.*) *nigrocellula* sp. nov., differing in several important details.

Nasus short and stout. Antennæ with scape brownish yellow. Squama with setæ. Wings with cell Sc light yellow, concolorous with cell C; outer two-thirds of cell 1st M_2 abruptly darkened; cell M_1 conspicuously pale; dark seam along m-cu scarcely invading cell M_4; cell 2d A uniformly suffused with pale brown.

Compared with *T.* (*S.*) *continuata* Brunetti, which likewise has a group of setæ on the squama, the present fly differs conspicuously in the coloration of the antennæ and body, and in the venation and wing pattern, especially the much longer Rs, which is somewhat longer than m-cu and nearly twice as long as R_{2+3}; elongate cell 1st M_2, with the second section of vein M_{1+2} nearly two-thirds as long as vein M_1; and in the broader cell M_4.

Habitat.—Kashmir.

Holotype, female, Kaj-Nag Range, altitude 8,000 feet, June 1, 1934 (*Miss Hutchinson*).

TIPULA (LUNATIPULA) TRIALBOSIGNATA sp. nov. Plate 1, fig. 6; Plate 2, figs. 30, 31.

Antennæ bicolorous; mesonotal præscutum with ground color yellow pollinose, narrowly or obsoletely striped with darker yellow; wings grayish, variegated with darker gray and whitish areas, including a broad, incomplete, poststigmal fascia; a dark spot in cell Cu, preceded and followed by whitish areas; abdomen yellow, in the female with the tergites narrowly trilineate with darker yellow; male hypopygium with the basistyle produced caudad into a subobtuse chitinized point; eighth sternite with caudal brush of long yellow setæ.

Male.—Length, about 15 millimeters; wing, 15.5.

Female.—Length, about 20 millimeters; wing, 18.5.

Frontal prolongation of head brownish yellow above, darker laterally; nasus distinct. Antennæ (male) of moderate length, if bent backward extending to the wing root or shortly beyond; scape and pedicel yellow; flagellar segments bicolorous, brownish black basally, the outer end yellow, narrowly so on the first flagellar segment; outer flagellar segments more uniformly darkened. Head brownish yellow.

Mesonotal præscutum with the ground color yellow pollinose, with four narrow brown stripes that represent the lateral stripes and posterior outer borders of the usual median stripe; in male,

præscutal stripes obsolete or nearly so; scutal lobes more or less variegated with darker color; scutellum and mediotergite yellow. Halteres yellow, the knobs infuscated. Legs with the coxæ and trochanters yellow; femora obscure yellow to brownish yellow, the tips narrowly dark brown, preceded by a slightly clearer, more yellowish ring; tibiæ and basitarsi obscure yellow, the tips narrowly dark brown; outer tarsal segments dark brown. Wings (Plate 1, fig. 6) with the ground color grayish, variegated by pale and darker areas; the white markings include one before stigma; a broad poststigmal fascia, extending from margin to cell 1st M_2, and an obliterative streak across base of cell 1st M_2, extending from cell R into bases of cells M_3 and M_4, usually disconnected from the poststigmal fascia but, in cases, confluent; additional white areas on either side of a dark cloud in cell Cu; the dark areas include the stigma and a confluent area along cord, together with the dark spot in cell Cu above described; narrow and less evident dark seams on posterior cord and outer end of cell 1st M_2; veins brown to brownish yellow. Macrotrichia of veins small and relatively sparse; squama with sparse setæ. Venation: R_{1+2} entire; petiole of cell M_1 shorter than m.

Abdomen entirely yellow or orange-yellow; in female, with indications of three narrow brown tergal stripes. Male hypopygium (Plate 2, fig. 30) with the tergite chiefly separated from the sternite. Ninth tergite (Plate 2, fig. 31, 9*t*) extensive, the caudal margin terminating in two small acute points that are separated from one another by a very shallow emargination; as in *Vestiplex*, these points are the dorsal manifestation of a tergal saucer that has swung caudad and ventrad so as to lie on the lower surface of the tergite. Basistyle, *b*, entire, the outer end produced into a subobtuse chitinized point; ventral portion with an oval lobe set with long yellow setæ. Outer dististyle slender, pale, from a slightly enlarged base. Inner dististyle, *id*, with the outer margin heavily blackened and corrugated. Eighth sternite, 8*s*, with a dense brush of long yellow setæ but without lobes or other armature. Ovipositor with the cerci rather stout, smooth-margined.

Habitat.—Kashmir.

Holotype, male, Kaj-Nag Range, altitude 9,000 feet, June 3, 1934 (*Miss Hutchinson*). Allotopotype, female. Paratopotypes, 1 male, 1 female.

The darkened cloud in cell Cu is suggestive of the condition found in many species of *Acutipula* and other subgeneric groups of *Tipula*, but the present fly seems correctly referrable to *Luna-*

tipula. The wing pattern and the structure of the male hypopygium, especially the basistyle, readily separate the species from other described Himalayan members of the genus.

TIPULA (OREOMYZA) ARISANENSIS Edwards. Plate 2, figs. 32, 33.

Tipula arisanensis EDWARDS, Ann. & Mag. Nat. Hist. IX 8 (1921) 109–110.

The types, two females, were from Arisan, Formosa, collected May 24, 1917, by Shiraki. One male, from Pianan-ambu, Formosa, May 11, 1932, collected by Gressitt, seems undoubtedly to belong to this species and is described herewith as allotype.

The male hypopygium is of rather remarkable construction, much as in *Tipula foliacea* Alexander, yet with the details quite distinct.

Ninth tergite (Plate 2, fig. 32) very large and massive, fused with the sternite on basal half, the outer half indicated by a gently curved suture; lateral tergal arms produced into powerful flattened blades that extend caudad about as far as the level of the tips of the inner dististyle; median area of tergite with two low, triangular points. Viewed from the side (Plate 2, fig. 33) the tergal blades are seen to be strongly decurved, narrowed outwardly, with a shallow notch close to tip.

Male.—Length, about 9.5 millimeters; wing, 11.5.

Allotype, male, Pianan-ambu, Formosa, May 11, 1932 (*Gressitt*).

In *T. foliacea* the tergal blades are much longer, extending far beyond the level of the dististyles; the intermediate lobes of the tergite are obtusely rounded.

TIPULA GRESSITTI sp. nov. Plate 1, fig. 7; Plate 2, fig. 34.

General coloration gray, the præscutum with four dark brownish gray stripes; antennæ (male) relatively elongate, bicolorous; front silvery white; tips of femora broadly black; wings gray, variegated by yellow and darker areas; male hypopygium very small and of simple structure; ovipositor with short, fleshy valves.

Male.—Length, 9 to 10 millimeters; wing, 13; antenna, 4.

Female.—Length, about 9 to 10 millimeters; wing, 11.

Frontal prolongation of head dark brown, sparsely pruinose; nasus long and conspicuous, tufted with black setæ; palpi black. Antennæ (male) elongate, the thirteenth segment microscopic; antenna approximately as long as the combined head and thorax; scape and pedicel yellow; first flagellar segment yellow, weakly darkened at base; succeeding segments conspicuously bicolorous,

the basal enlargement black, the remainder yellow; outer segments more obscure but all except the twelfth retaining the bicolorous appearance; verticils a little shorter than the segments. Head with the front silvery white; anterior vertex and orbits yellow; disk of vertex more infumed, variegated by an elongate brown area on either side of the median furrow, the inner ends of the marks dilated, the narrow outer portion paralleling the orbits; an additional smaller dark spot immediately behind each antennal fossa.

Mesonotum gray, the præscutum with four dark brownish gray stripes, the intermediate pair separated only by a capillary line; scutum dark brownish gray, the anterior portion darkened by a posterior prolongation of the lateral præscutal stripes; posterior sclerites of mesonotum blackened, sparsely pruinose. Pleura black, heavily pruinose, more heavily so on ventral sternopleurite; dorsopleural membrane obscure yellow. Halteres chiefly pale yellow, darkened only by abundant black setæ near base of club. Legs with coxæ brownish gray, paler apically; trochanters yellow; femora obscure brownish yellow, the tips broadly and conspicuously blackened; tibiæ and tarsi brownish black to black; tibial spur formula apparently 1–1–2. Wings (Plate 1, fig. 7) with the ground color gray, variegated by brown and yellow areas; prearcular region and cell Sc yellow; cell C uniformly infuscated; stigma dark brown; a distinct brown cloud on anterior cord; the yellow color includes areas before and beyond stigma; near base and near outer end of cell M; near outer ends of cells M_1 to M_4, inclusive; most of cell Cu and areas in cell 1st A; cell 2d A more uniformly darkened; veins brown. Squama naked; macrotrichia of veins relatively long and numerous. Venation: R_{1+2} entire; petiole of cell M_1 variable in length, in the allotype nearly lacking so that cell M_1 is subsessile; cell 2d A relatively narrow.

Abdomen brown, the basal two tergites variegated with obscure yellow; lateral margin of second tergite blackened; hypopygium dark. Male hypopygium (Plate 2, fig. 34) very small and of simple structure, among the most generalized so far discovered in the genus. Ninth tergite separated from the fused sternite-basistyle; suture between basistyle and sternite indicated beneath, the former region extensive. Ninth tergite, 9*t*, transverse, the caudal margin with a broad shallow emargination, the lateral lobes obtuse, with abundant normal setæ. Outer dististyle, *od*, broadly flattened, the apex obtuse. Inner disti-

style, *id*, simple, the apical beak very long and slender. Eighth sternite simple, weakly sheathing, the outer end membranous but with abundant setæ. Ovipositor with short fleshy valves.

Habitat.—Formosa.

Holotype, male, Arisan, altitude 7,640 feet, May 24, 1934 (*Gressitt*). Allotopotype, female. Paratopotypes, 2 males, 1 female.

Tipula gressitti is named in honor of the collector, who has secured many interesting Tipulidæ in the high mountains of Formosa. I cannot place the fly in any of the now numerous subgenera of the vast genus *Tipula*. The Eastern Nearctic *Nobilotipula* Alexander has an ovipositor of somewhat similar structure, but the present fly scarcely seems to be consubgeneric. In its general appearance, especially in body coloration and wing coloration, the species bears a considerable resemblance to several other regional members of the subgenera *Acutipula, Lunatipula, Oreomyza,* and *Vestiplex,* but in the details of structure is very distinct.

NEPHROTOMA ATROLATERA sp. nov. Plate 1, fig. 8.

Mesonotal præscutum with the central portion largely covered by a shield-shaped pearly area that is margined in front and on sides by a velvety-black border; scutellum and mediotergite light yellow; pleura yellow; abdominal tergites yellow, trilineate with dark brown; sternites uniformly orange-yellow.

Female.—Length, about 14 millimeters; wing, 13.5.

Frontal prolongation of head light sulphur yellow; nasus with long black setæ; mouth parts more fulvous; palpi dark brown. Antennæ with basal segments brownish yellow, the flagellum passing into darker brown. Front light sulphur yellow; posterior portion of head dark orange, more yellowish behind but without evident occipital brand.

Pronotum entirely light sulphur yellow. Mesonotal præscutum with three confluent pearly stripes, the shield-shaped area thus formed bordered by velvety black, including a narrow cephalic border, broader lateral margins, and a deep triangular invasion of the humeral field; scutum with centers of lobes pearly, narrowly but completely bordered by velvety black; median region of scutum yellow; scutellum and mediotergite light sulphur yellow, with a narrow transverse dark line separating the two; mediotergite with numerous short black setæ. Anterior lateral pretergites sulphur yellow. Pleura yellow. Halteres dusky, the knobs yellow. Legs with the coxæ and trochanters light yellow; femora and tibiæ light brown, the tips weakly darkened; tarsi

black. Wings (Plate 1, fig. 8) somewhat teneral in the unique type, subhyaline; stigma and a seam along the cord narrowly darker; veins brown. Venation: Cell M_1 broadly sessile; m-cu uniting with M just before departure of vein M_4.

Abdominal tergites yellow, conspicuously trilineate with dark brown, the stripes entire or virtually so; sternites orange-yellow, the caudal borders of the segments restrictedly paler yellow.

Habitat.—Formosa.

Holotype, female, Hassensan, altitude 4,875 feet, June 22, 1934 (*Gressitt*).

The present fly is amply distinct from other described species of the genus in the pattern of the mesonotal præscutum and scutum. The presence of short, dense, black setæ on the postnotal mediotergite reminds one of *Nephrotoma medipubera* Edwards (eastern Java), which is otherwise a very different fly.

DOLICHOPEZA (NESOPEZA) LUGUBRIVESTIS sp. nov. Plate 1, fig. 9; Plate 2, fig. 35.

General coloration dark brown, the præscutum with three more-glabrous, somewhat nacreous stripes; pleura dark brown; legs black, only the outer tarsal segments restrictedly yellowish white; wings with the ground color strongly blackened; stigma darker, preceded and followed by whitish areas; Rs short, a little less than m-cu; male hypopygium with the lateral lobes of the tergal plate broadly rounded; gonapophyses long and conspicuous, the tips acute.

Male.—Length, about 12 millimeters; wing, 13.

Frontal prolongation of head brown; palpi black. Antennæ of moderate length, if bent backward extending approximately to base of abdomen; scape and pedicel brownish yellow, flagellum black; verticils abundant, the longest on the upper face and well scattered over the length of the segment. Front and vertical tubercle brownish yellow, the posterior portions of head more uniformly dark brown.

Mesonotal præscutum dull dark brown, with three more-glabrous, nacreous stripes, the median one broad and well defined, the lateral pair less clearly delimited; posterior sclerites of notum dark brown, the median area of scutum and mediotergite paler. Pleura dark brown, the dorsopleural region paler. Halteres with the stem dusky, narrowly obscure yellow at base, the knobs dark brown. Legs with the coxæ dark brown, paler apically; trochanters obscure yellow; femora and tibiæ black, the former restrictedly paler at base; tarsi black basally, the outer segments narrowly and restrictedly yellowish white, more ex-

tensively so on the posterior legs where the brightening involves about the outer two-thirds of the second tarsal segment and the succeeding segments; on the anterior legs the bright color of the tarsi is even more obscured. Wings (Plate 1, fig. 9) with the ground color strongly blackened, the stigma even darker, preceded and followed by whitish areas; a restricted darkened cloud on anterior cord; veins brownish black. Venation: Rs short, a little less than m-cu; medial forks of moderate depth.

Abdominal tergites dark brown, the incisures restrictedly more blackened, outer segments, including hypopygium, more uniformly blackened. Male hypopygium (Plate 2, fig. 35) with the lateral lobes of the tergal plate, 9*t*, broad, their caudal margins broadly rounded. Ædeagus and subtending apophyses, *g*, very long and conspicuous, as illustrated; apophyses at base with a small cylindrical lobe but beyond this point simple, the tips acute.

Habitat.—Formosa.

Holotype, male, Arisan, altitude 7,475 feet, May 25, 1934 (*Gressitt*).

Dolichopeza (*Nesopeza*) *lugubrivestis* is most nearly allied to *D.* (*N.*) *idiophallus* (Alexander) and *D.* (*N.*) *tarsalba* (Alexander), differing from both in the coloration of the body and wings, the venation, especially the short Rs, and the details of structure of the male hypopygium.

LIMONIINÆ

LIMONIINI

LIMONIA (LIMONIA) SUBHOSTILIS sp. nov. Plate 1, fig. 10; Plate 3, fig. 36.

General coloration brown, the præscutal stripes not or scarcely indicated; antennæ black throughout; each of the outer flagellar segments with a single very long verticil, these unilaterally arranged; outer flagellar segments gradually increasing in length to the last; halteres elongate; legs brown, the femoral tips not brightened; wings broad, suffused with gray; stigma lacking; Sc_2 and R_{1+2} both very long; male hypopygium with a single dististyle, this terminating in a slender apical beak.

Male.—Length, about 10 millimeters; wing, 11.8.

Rostrum brown; palpi dark brown. Antennæ dark brown throughout; outer flagellar segments with a single verticil of unusual length, placed on outer face near base, this seta approximately twice the length of the segment bearing it; remain-

ing verticils relatively small and insignificant; flagellar segments beyond midlength of organ becoming progressively more elongate, the terminal segment longest. Head dark brown.

Mesonotum almost uniformly medium brown, the surface polished; præscutal stripes not or scarcely defined, the lateral pair indicated chiefly by a slightly darkened lateral portion. Pleura testaceous-brown. Halteres unusually long and slender, dark brown. Legs with the coxæ and trochanters brown; remainder of legs medium brown, the femoral bases not or scarcely brightened; claws with a long slender spine at near midlength, with a second smaller, more basal tooth. Wings (Plate 1, fig. 10) broad, almost uniformly suffused with gray; stigma not or scarcely indicated; veins brown. Macrotrichia of veins relatively long and conspicuous, on all longitudinal veins beyond cord, extreme distal end of vein 1st A and outer two-thirds of 2d A. Venation: Sc_2 very long, as in *hostilis*, several times as long as Sc_1 and considerably longer than R_2; R_{1+2} elongate; cell 1st M_2 wide; m-cu close to fork of M.

Abdomen dark brown; hypopygium somewhat paler. Male hypopygium (Plate 3, fig. 36) with the tergite, 9*t*, narrowed outwardly, the apex abruptly thin and glabrous, with a U-shaped median notch, the lateral lobes thus formed low and subtruncate. Dististyle, *d*, single, its apical beaklike portion unusually slender. Gonapophyses, *g*, with the mesal-apical lobe broad. Ædeagus broad at apex.

Habitat.—Formosa.

Holotype, male, Arisan, altitude 7,312 feet, May 27, 1934 (*Gressitt*).

The nearest described ally of the present fly is undoubtedly *Limonia* (*Limonia*) *hostilis* Alexander (Szechwan-Tibet border, at high altitudes), which differs conspicuously in the abruptly yellow tips of the femora and in slight details of venation and body coloration.

LIMONIA (DICRANOMYIA) TRANSFUGA sp. nov. Plate 1, fig. 11.

Mesonotal præscutum with a broad median brown stripe, the lateral borders of the sclerite broadly golden yellow; pleura pruinose; knobs of halteres dark brown; femora blackened at tips, the fore femora more extensively so; wings tinged with yellow, sparsely patterned with brown; abdomen with basal six segments orange-yellow, the remaining outer segments black.

Female.—Length, about 9.5 millimeters; wing, 10.

Rostrum brown, sparsely pruinose; palpi black. Antennæ black throughout; basal flagellar segments subglobular, the outer segments oval to elongate-oval. Head brownish gray.

Mesonotum with a broad median brown stripe, the lateral portions of the sclerite broadly and conspicuously golden-yellow pollinose; lateral præscutal stripes scarcely indicated; scutal lobes variegated with darker yellow; mediotergite black, sparsely pruinose, the lateral portions paling to yellow. Pleura yellow, the mesopleura and pleurotergite heavily pruinose. Halteres with basal portion of stem yellow, the outer portion and knob dark brown. Legs with the coxæ and trochanters yellow; fore femora black, the basal third yellow; remaining femora yellow, the tips more narrowly blackened, not including more than the distal fourth or fifth; tibiæ and tarsi dark brown to brownish black. Wings (Plate 1, fig. 11) with a strong yellow tinge, the costal border and outer radial field more-saturated brownish yellow; stigma and narrow seams along cord and outer end of cell 1st M_2 dark brown; a brownish seam along vein Cu in cell M; veins brownish yellow, darker in the clouded areas. Venation: Sc_1 ending a short distance beyond origin of Rs, Sc_2 some distance before this origin; free tip of Sc_2 and R_2 in transverse alignment; m-cu close to fork of M, subequal to or a little longer than distal section of Cu_1; anal veins at origin subparallel or very slightly convergent.

Abdomen with the basal six segments orange-yellow, the remaining segments black. Ovipositor with the cerci relatively short and unusually slender, gently upcurved to the acute tips, a little longer than the hypovalvæ.

Habitat.—Kashmir.

Holotype, female, Kaj-Nag Range, altitude 9,000 feet, May 30, 1934 (*Miss Hutchinson*).

Limonia (*Dicranomyia*) *transfuga* is quite distinct from other regional members of the subgenus, superficially bearing a certain resemblance to *L.* (*D.*) *baileyi* (Edwards), of Tibet, yet entirely distinct.

PEDICIINI

DICRANOTA (RHAPHIDOLABIS) SUBSORDIDA sp. nov. Plate 1, fig. 12; Plate 3, fig. 37.

General coloration gray, the præscutum with a darker median stripe; antennæ black throughout; knobs of halteres infuscated; legs brownish black; wings grayish subhyaline, the oval stigma brown; R_{2+3+4} approximately twice the basal section of R_5; abdominal tergites dark brown, hypopygium brownish yellow;

male hypopygium with the caudal margin of tergite transverse, each outer angle produced into a low setiferous lobe; interbase widely expanded on proximal portion.

Male.—Length, about 5 millimeters; wing, 6.2.

Rostrum gray; palpi black. Antennæ short, black throughout, the scape a little pruinose. Head gray.

Mesonotum gray, the præscutum with a darker, plumbeous gray, median stripe. Pleura gray. Halteres pale, the knobs infuscated. Legs with the coxæ pruinose on outer face, the inner face pale; trochanters yellow; femora brownish black, the bases restrictedly pale; tibiæ and tarsi brownish black. Wings (Plate 1, fig. 12) grayish subhyaline; stigma oval, brown; veins dark brown. Venation: R_{2+3+4} approximately twice the basal section of R_5; R_2 transverse; cell M_1 unusually small, the inclosing branches divergent; m-cu about one-half its length beyond fork of M.

Abdominal tergites dark brown; sternites somewhat paler, slightly pruinose; hypopygium brownish yellow. Male hypopygium (Plate 3, fig. 37) with the tergite, 9*t*, transverse across caudal margin, each lateral portion produced into a low, rounded, setiferous lobe. Dististyle, *d*, with the outer blade elongate, flattened, a little expanded on outer end and here provided with several setæ. Interbase, *i*, a yellow blade, very widely expanded on proximal portion, gradually narrowed outwardly, the tip narrowly obtuse.

Habitat.—Kashmir.

Holotype, male, Kaj-Nag Range, altitude 8,000 feet, May 18, 1934 (*Miss Hutchinson*).

The species is most readily told from *Dicranota* (*Rhaphidolabis*) *sordida* (Brunetti) by the black antennæ and almost uniformly darkened legs.

DICRANOTA (RHAPHIDOLABIS) UNINEBULOSA sp. nov. Plate 1, fig. 13; Plate 3, fig. 38.

General coloration gray, the præscutum without evident stripes; antennal flagellum black; knobs of halteres weakly darkened; femora brownish yellow, the tips weakly darkened; wings subhyaline, stigma brown; a small brown cloud on anterior cord; R_{2+3+4} fully one-half longer than basal section of vein R_5; abdomen brown, sparsely pruinose, the extreme caudal margins of the segments pale; hypopygium yellow; male hypopygium with the interbase curved, the apex flattened and having the margin microscopically serrulate.

Male.—Length, about 6.5 millimeters; wing, 7.5.

Rostrum gray; palpi dark. Antennæ short; scape and pedicel brownish black, flagellum black; antennæ 15-segmented; flagellar segments short-oval to subglobular. Head dark gray.

Mesonotum gray, the præscutum without evident stripes. Pleura gray. Halteres pale, the knobs weakly darkened. Legs with the coxæ pale, gray pruinose; femora brownish yellow, the tips weakly darkened; tibiæ pale brown, the tips, together with the tarsi, more brownish black. Wings (Plate 1, fig. 13) subhyaline; stigma brown; a small but distinct brown cloud on anterior cord; veins brown. Venation: R_{2+3+4} fully one-half longer than the basal section of vein R_5; R_2 transverse or nearly so; M almost in direct alignment with M_{1+2}, the basal section of the latter lacking or virtually so; m-cu about one-half its length beyond fork of M.

Abdomen brown, sparsely pruinose; extreme caudal margins of segments pale; hypopygium yellow. Male hypopygium with the interbase (Plate 3, fig. 38, *i*) curved, the apex flattened, its margin microscopically serrulate.

Habitat.—Kashmir.

Holotype, male, Kaj-Nag Range, altitude 8,000 feet, May 26, 1934 (*Miss Hutchinson*).

Dicranota (*Rhaphidolabis*) *uninebulosa* is readily told from the other Himalayan species of the subgenus by the large size and the pattern of the wings.

DICRANOTA (RHAPHIDOLABIS) PALLIDITHORAX sp. nov. Plate 1, fig. 14; Plate 3, fig. 39.

General coloration of thorax pale brownish yellow; antennæ dark brown, the scape brownish yellow; knobs of halteres darkened; wings subhyaline, the stigmal area faintly darker; R_{2+3+4} about one-half longer than the basal section of vein R_5; R_2 oblique; male hypopygium with the interbase on mesal face at near midlength produced into a spinous point.

Male.—Length, about 7 millimeters; wing, 7.8.

Female.—Length, about 8 millimeters; wing, 8.5.

Rostrum brown, paler laterally; palpi brown. Antennæ short, 15-segmented; scape brownish yellow, remaining segments dark brown. Head light gray throughout.

Pronotum and mesonotum, together with the pleura, entirely pale brownish yellow to yellow, without markings. Halteres pale, the knobs darkened. Legs with the coxæ and trochanters yellow; femora brownish yellow, clearer yellow basally, darker outwardly; tibiæ and tarsi dark brown. Wings (Plate 1, fig.

14) subhyaline, iridescent; stigmal area faintly darker, pale brown; veins brown. Venation: Rs of moderate length; R_{2+3+4} about one-half longer than the basal section of vein R_5; R_2 oblique, exceeding one-half R_{1+2}; m-cu about its own length beyond the fork of M.

Abdominal tergites dark brown, the caudal borders of the segments very narrowly pale; basal sternites obscure yellow, the outer segments somewhat more darkened; hypopygium chiefly yellow. Male hypopygium with the interbase (Plate 3, fig. 39, *i*) of characteristic shape, on mesal face at near midlength produced into an acute spinous point, the apical portion slender, narrowed before the oval distal end.

Habitat.—Kashmir.

Holotype, male, Kaj-Nag Range, altitude 8,000 feet, May 22, 1934 (*Miss Hutchinson*). Allotopotype, female.

Dicranota (*Rhaphidolabis*) *pallidithorax* is very distinct from the other Asiatic species of the subgenus so far described in the major size, the pale coloration of the thorax, and the structure of the interbase of the male hypopygium.

DICRANOTA (AMALOPINA) FUMICOSTATA sp. nov. Plate 1, fig. 15; Plate 3, fig. 40.

Mesonotal præscutum yellow, with a median darker stripe; posterior sclerites of mesonotum darkened; legs yellow; wings yellow, the costal border to apex broadly infumed, including all of cells C and Sc; smaller darker areas on certain of the veins and crossveins; no supernumerary crossvein in cell R_1; R_{2+3+4} short; cell 1st M_2 closed; male hypopygium with both the tergal arms and the interbases appearing as stout flattened blades that terminate in acute spines.

Male.—Length, about 5.5 millimeters; wing, 6.5.

Rostrum and palpi brownish black. Antennæ with the scape brownish black; pedicel brownish yellow; flagellum light yellow, only the outer two or three segments more darkened. Head brown; eyes relatively large and protuberant.

Cervical sclerites and central portion of pronotum dark brown. Mesonotal præscutum yellow, with a more brownish median stripe; lateral stripes narrow and scarcely evident; posterior sclerites of mesonotum darker brown, this color including the posterior border of the pleurotergite. Remainder of pleurotergite and all of the pleura pale yellow. Halteres with the stem yellow, its outer end and the knob infuscated. Legs with the coxæ and trochanters pale yellow; remainder of legs light yellow,

only the last tarsal segment darkened. Wings (Plate 1, fig. 15) with the ground color yellowish, the costal border to apex broadly infumed, the darkening including all of cells C and Sc, together with the basal third of cell R; restricted darker brown areas at origin of Rs, along cord, R_2, outer end of cell 1st M_2 and fork of M_{1+2}; veins pale, darker in the infumed areas. Venation: Sc_2 lying at near three-fifths the distance between arculus and origin of Rs; no supernumerary crossvein in cell R_1; cell R_3 short-petiolate, R_{2+3+4} being represented by an element that is subequal to or shorter than the basal section of R_5; cell 1st M_2 closed; m-cu about one-half its length beyond the fork of M.

Abdominal tergites brown, the sternites paler; abdomen darker apically. Male hypopygium (Plate 3, fig. 40) with the lateral arms of the tergite, 9*t*, appearing as powerful erect rods, the tip incurved to a short acute spine; median area of tergite slightly convex, with abundant setæ. Interbases, *i*, large and powerful, appearing as sinuous yellow blades, the tip of each narrowed into an acute spine. Dististyle unusually simple, the outer lobe with the usual close-set spines, the inner blade with numerous setæ.

Habitat.—Formosa (north).

Holotype, male, Urai, altitude 1,500 feet, April 1, 1932 (*Gressitt*).

Dicranota (*Amalopina*) *fumicostata* is very different from the other regional species of the subgenus, the distinctions being best shown by the accompanying key to the five species now known from the Japanese Empire.

Key to the Japanese species of Amalopina.

1. Cell 1st M_2 open by atrophy of m; fore and middle femora brownish black, the posterior femora and remainder of all legs light yellow (*gibbera* and races) ..... 2.
   Cell 1st M_2 closed; legs yellow ..... 3.
2. Wings (male) broad, widest opposite end of vein 2d A. (Japan.) *gibbera gibbera* Alexander.
   Wings (male) narrow, of approximately equal width along the central third of length. (Japan.) ..... *gibbera karafutonis* Alexander.
3. No supernumerary crossvein in cell R_1; outer ends of radial cells uniformly infumed. (Formosa.) ..... *fumicostata* sp. nov.
   A supernumerary crossvein in cell R_1; outer ends of radial cells clear or with darkenings at ends of veins only ..... 4.
4. Cells C and Sc undarkened. (Japan.) ..... *dicranotoides* Alexander.
   Cell C, and usually Sc also, strongly darkened on basal half ..... 5.

5. Outer ends of all longitudinal veins with brown spots and dots. (Siberia and Korea.) ..... *siberica* Alexander.
Outer ends of longitudinal veins undarkened. (Formosa.)
delectata Alexander.

It may be noted that all of the above species (excepting *siberica*, a male of which is not available to me at this time) show marked distinctions in the structure of the male hypopygium.

ERIOPTERINI

GONOMYIA (GONOMYIA) JUSTA sp. nov. Plate 1, fig. 16; Plate 3, fig. 41.

Belongs to the *tenella* (*subcinerea*) group; allied to *G.* (*G.*) *nebulicola;* antennæ black throughout; mesonotum grayish brown, restrictedly variegated with yellow; pleura sulphur yellow, variegated by reddish brown; legs dark brown; R_{2+3+4} strongly arcuated; male hypopygium with the outer lobe of basistyle relatively short, only a little longer than the dististyle, the latter broadly flattened, bispinous; blackened appendage of phallosome not spinous.

Male.—Length, about 4.2 millimeters; wing, 5.

Rostrum yellow; palpi brownish black. Antennæ black throughout; outer flagellar segments very slender, almost setaceous, the basal ones more enlarged. Head gray.

Pronotum and anterior pretergites light yellow. Mesonotum uniformly dark grayish brown, the humeral region and sides of præscutum restrictedly yellow; median area of scutum obscure yellow; posterior lateral portions of scutal lobes and the broad posterior border of scutellum dark yellow. Pleura light sulphur yellow, variegated by reddish or reddish brown on anepisternum and more extensively on ventral sternopleurite and meron. Halteres dusky, the base of stem light yellow. Legs with the coxæ reddish or yellow, the fore pair darker; trochanters obscure yellow; remainder of legs dark brown. Wings (Plate 1, fig. 16) uniformly tinged with gray, the stigma pale brown; veins dark brown. Venation: Sc_1 ending shortly beyond origin of Rs, Sc_2 opposite this origin; R_{2+3+4} strongly arcuated; basal section of R_5 reduced; cell 1st M_2 relatively small; m-cu at fork of M.

Abdominal tergites dark brown, the sternites and hypopygium more brownish yellow. Male hypopygium (Plate 3, fig. 41) with the outer lobe of basistyle, *b*, relatively short, only a little longer than the dististyle. Dististyle, *d*, broadly flattened, bearing two unequal spines on outer margin, the outermost long and

gently curved, with a single seta at base; second spine in axil of the first; no carina connecting the inner spine with apex of style, as is the case in *nebulicola*. Phallosome, *p*, with a single blackened appendage, this subobtuse at apex, not spinous.

Habitat.—Java.

Holotype, male, Mount Malabar, altitude about 4,000 feet, May 26, 1929 (*Mackerras*). Paratopotype, male.

Type in the National Collection, Federal Capital Territory, Canberra.

The nearest described ally of the present fly is undoubtedly *Gonomyia* (*Gonomyia*) *nebulicola* Alexander (Mindanao), which differs chiefly in the structure of the dististyle and phallosome of the male hypopygium, as contrasted above.

GONOMYIA (LIPOPHLEPS) TORAJA sp. nov. Plate 1, fig. 17; Plate 3, fig. 42.

Mesonotum dark gray, the scutellum obscure yellow, pruinose; thoracic pleura with a silvery white longitudinal stripe; knobs of halteres yellow; femora obscure yellow, the tips broadly blackened; wings with a grayish tinge, the ground color vaguely brightened by paler areas; veins along cord darkened; basal section of R_5 long; abdomen dark brown; male hypopygium with two dististyles, the outer a long simple black rod; inner style profoundly bifid, its outer arm fusiform, clothed with long conspicuous setæ.

Male.—Length, about 3 millimeters; wing, 3.2.

Rostrum and palpi black. Antennæ black; flagellar segments (male) with very long verticils. Head brownish yellow, the center of vertex darker.

Sides of pronotum and the anterior lateral pretergites whitish, the posterior pretergites more silvery. Mesonotal præscutum dark gray; pseudosutural foveæ black; scutum dark gray, including the median area; scutellum obscure yellow, sparsely pruinose, the base darkened; mediotergite dark, heavily pruinose. Pleura dark, including the dorsopleural membrane; a conspicuous silvery longitudinal stripe extending from behind the fore coxæ to the base of abdomen; indications of a second, much less distinct, obscure yellow stripe above the first, involving the pteropleurite, pleurotergite, and cephalic-lateral portions of the mediotergite. Halteres dusky, the knobs yellow apically. Legs with the coxæ dark brown, the fore coxæ pale on outer face; trochanters brownish yellow; femora obscure yellow, the tips broadly black (certain of the femoral tips are broken and it cannot be affirmed as to whether or not certain of these areas

are slightly subterminal); tibiæ yellow, the tips very narrowly darkened; tarsi broken. Wings (Plate 1, fig. 17) with a grayish tinge, the ground color vaguely brightened by paler areas, chiefly before origin of Rs and beyond stigma; stigmal area very faint; a darkened area along cord, indicated chiefly by the darker veins; veins pale, except as described. Costal fringe long. Venation: Sc_1 ending shortly before the origin of Rs; basal section of R_5 long; m-cu shortly before fork of M.

Abdomen, including hypopygium, dark brown. Male hypopygium (Plate 3, fig. 42) with the basistyle, *b*, unproduced at outer end. Two dististyles, the outer, *od*, a long, simple, blackened rod, curved and gently sinuous to the acute tip; inner style, *id*, profoundly bifid, its outer arm a yellow fusiform structure that terminates in an acute spine, the surface with abundant setæ; inner arm shorter, with marginal setæ. Phallosome, *p*, consisting of two pairs of broadly flattened, superimposed plates.

Habitat.—Central Celebes (District Bontoe Batoe).

Holotype, male, Latimodjong Mountains, altitude 3,800 feet, May 15, 1931 (*Clagg*).

Gonomyia (*Lipophleps*) *toraja* is named from an aboriginal tribe inhabiting central Celebes. The species is readily told from all other regional species of the subgenus by the structure of the male hypopygium. This latter has a structure generally like that of *G.* (*L.*) *kertesziana* Alexander (northeastern New Guinea), but the details are entirely different.

GONOMYIA (LIPOPHLEPS) TOALA sp. nov. Plate 1, fig. 18; Plate 3, fig. 43.

General coloration of notum dark brownish gray, the lateral border of the præscutum conspicuously lighter gray; pleura striped longitudinally with whitish; halteres yellow; femora and tibiæ brownish yellow; wings yellowish gray; stigmal area faintly indicated; Rs angulated, and, in cases, short-spurred at origin; male hypopygium with three dististyles, the intermediate one profoundly bifid; phallosome consisting of flattened pale plates, without blackened points or spines.

Male.—Length, about 2.8 to 3 millimeters; wing, 3.4 to 3.6.

Rostrum and palpi black. Antennæ black, the pedicel more or less brightened; verticils (male) unusually long and delicate. Head brownish gray, more or less variegated by paler gray.

Mesonotum chiefly dark brownish gray, the lateral border of the præscutum conspicuously lighter gray; posterior sclerites of notum, including the scutellum, gray. Pleura brown, with a broad whitish longitudinal stripe extending from and including

the fore coxæ to the base of abdomen, this stripe narrowly bordered both above and beneath by still darker brown. Halteres yellow. Legs with the coxæ brown, the fore coxæ whitish, as described; trochanters obscure yellow; remainder of legs brownish yellow, the outer tarsal segments more blackened. Wings (Plate 1, fig. 18) with a yellowish gray tinge, the prearcular and costal portions a little clearer yellow; stigmal area faintly indicated; veins pale. Venation: Sc of moderate length, Sc_1 ending a short distance before origin of Rs; Rs angulated and sometimes short-spurred at origin; m-cu a short distance before fork of M.

Abdomen dark brown, the pleural region paler; incisures, especially of the outer segments, a little brightened. Male hypopygium (Plate 3, fig. 43) with the apex of basistyle, *b*, unproduced. Three dististyles; outer, *od*, a slender, nearly straight rod, the apex narrowly blackened and gently curved into a spine, the outer margin with a series of microscopic setæ; intermediate style, *md*, profoundly bifid, the outer arm a little longer than the outer style, appearing as a flattened pale blade that is slightly arcuate; inner arm of this style much shorter, its apex a blackened spine; inner style, *id*, elongate-oval, with numerous setæ, none of which is evidently fasciculate. Phallosome, *p*, appearing as two flattened pale plates, with a slenderer median pale structure, the entire organ without blackened points or spines.

Habitat.—Central Celebes (District Bontoe Batoe).

Holotype, male, Latimodjong Mountains, altitude 3,800 feet, May 15, 1931 (*Clagg*). Paratopotype, male.

The specific name of this species, *toala*, is that of an aboriginal tribe. The fly is quite distinct from all other regional members of the subgenus in the structure of the male hypopygium, especially the profoundly bifid intermediate dististyle and the entirely pale phallosome.

LIPSOTHRIX KASHMIRICA sp. nov. Plate 1, fig. 19; Plate 3, fig. 44.

Thorax yellow; legs yellow, the tips of femora and narrow bases of tibiæ brownish black; wings whitish hyaline, without stigma; veins brown, the prearcular veins light yellow; abdominal tergites with a broad, continuous, dark brown, median stripe; male hypopygium with the interbase bearing two small acute spines on outer face before midlength.

Male.—Length, 7.5 to 8 millimeters; wing, 8 to 8.5.

Female.—Length, 8.5 to 9.5 millimeters; wing, 9 to 9.5.

Rostrum yellow, palpi pale. Antennæ with basal segments pale yellow, the outer ones passing into brown; the number of pale basal segments varies considerably in different specimens, in some cases involving several of the flagellar segments, as well as the scape and pedicel. Head brownish yellow.

Thorax entirely yellow. Halteres yellow. Legs yellow, the femoral tips and narrower tibial bases brownish black; tips of tibiæ narrowly darkened; outer tarsal segments infuscated. Wings (Plate 1, fig. 19) whitish hyaline, without stigmal or other markings; veins brown, the prearcular veins light yellow. Macrotrichia on all longitudinal veins beyond cord, including the outer ends of both anal veins, the amount on the latter veins variable in different specimens. Venation: m-cu at or close to fork of M; basal section of R_5 and r-m often angulated and weakly spurred.

Abdominal tergites with a broad, continuous, dark brown, median stripe, the lateral borders of the segments yellow, more widely so on the outer segments; seventh to ninth segments (male) uniformly blackened. Male hypopygium (Plate 3, fig. 44) with the interbase, *i*, long and sinuous, at base with a slender spinous point; on outer margin before midlength with two small acute spines.

Habitat.—Kashmir.

Holotype, male, Kaj-Nag Range, altitude 9,000 feet, May 30, 1934 (*Miss Hutchinson*). Allotopotype, female, altitude 8,000 feet, May 24, 1934. Paratopotypes, 15 of both sexes, altitude 8,000 feet, May 22 to 30, 1934.

Lipsothrix kashmirica is most nearly allied to *L. errans* (Walker), differing especially in the nearly hyaline wings and conspicuous, entire, median dark stripe on abdominal tergites. Edwards[2] has indicated the existence in Europe of no fewer than four species, of which three occur in Britain. Four other species are found in Japan and Formosa, but hitherto none had been recorded from the Asiatic mainland.

ORMOSIA HUTCHINSONÆ sp. nov. Plate 1, fig. 20; Plate 3, fig. 45.

Mesonotum gray, with scarcely indicated præscutal stripes; antennæ with scape and pedicel pale, flagellum black; thoracic pleura yellow; knobs of halteres dark brown, femora yellow, the tips broadly blackened; wings whitish subhyaline; stigma and

[2] Entomologist 63 (1930) 212.

very narrow seams along cord and outer end of cell 1st M_2 brown; cell 1st M_2 closed; anal veins divergent; abdominal tergites brownish black, the sternites and hypopygium yellow.

Male.—Length, 3.8 to 4.2 millimeters; wing, 4.5 to 5.

Female.—Length, about 5 to 5.5 millimeters; wing, 5.5 to 6.

Rostrum brownish yellow to yellow; palpi dark brown. Antennæ short; scape light yellow; pedicel brownish yellow; flagellum black; flagellar segments oval, the outer segments a little more elongate. Front light yellow; vertex light gray.

Mesonotum gray, the præscutal stripes not or scarcely defined; tuberculate pits black, conspicuous; lateral margins of præscutum paling to yellow; lateral pretergites light yellow. Pleura yellow to weakly infumed, contrasting with the notum. Halteres dark brown, the basal portion of stem yellow. Legs with the coxæ and trochanters light yellow; femora yellow, the tips broadly blackened; tibiæ brown, the tips blackened; tarsi black. Wings (Plate 1, fig. 20) whitish subhyaline; stigma and very narrow seams along cord and outer end of cell 1st M_2 brown; veins brown. Venation: Cell 1st M_2 closed; m-cu not far beyond the fork of M; anal veins divergent.

Abdominal tergites brownish black, sparsely pruinose; lateral borders of segments very narrowly pale; sternites and hypopygium light yellow. Male hypopygium (Plate 3, fig. 45) with the dististyle, *d*, apparently simple but deeply bifid, the outer arm a cylindrical blackened structure that terminates in a group of acute teeth; inner arm pale, broad-based, narrowed to a long slender point, the tip obtuse.

Habitat.—Kashmir.

Holotype, male, Kaj-Nag Range, altitude 8,000 feet, May 15, 1934 (*Miss Hutchinson*). Allotopotype, female. Paratopotypes, 15 of both sexes.

Ormosia hutchinsonæ is named in honor of Miss Vivien R. Hutchinson, to whom I am greatly indebted for many interesting Tipulidæ from Kashmir. The species is readily distinguished from all other Palæarctic species having cell 1st M_2 closed by the coloration of the body and peculiar conformation of the dististyle of the male hypopygium.

STYRINGOMYIA CELEBESENSIS sp. nov. Plate 1, fig. 21; Plate 3, fig. 46.

General coloration yellow; mesonotum, especially the scutum, with specially modified setæ; wings with vein R_3 nearly transverse; 2d A strongly curved to margin; male hypopygium with

apex of basistyle bispinous; outer arm of dististyle without median projection.

Male.—Length, about 5.3 to 5.5 millimeters; wing, 5.2 to 5.4.

Female.—Length, about 5 millimeters; wing, 4.5.

Rostrum brown; palpi dark brown, the terminal segment paler. Antennæ with the scape and pedicel dark beneath, more yellow above; flagellum yellow, the basal segments short and crowded. Head grayish brown; setæ large and conspicuous.

Pronotum whitish, with conspicuous setæ. Mesonotal præscutum yellowish brown, vaguely lined with darker brown; notal setæ conspicuous, especially those of the scutum, much as in *S. ensifera;* mediotergite pale. Pleura testaceous-yellow. Halteres yellow. Legs with the coxæ and trochanters yellow; remainder of legs yellow, the femoral and tibial rings broad but diffuse, brown; fore femora with a group of strong black setæ at tip; tarsi pale, the tips of the segments narrowly darkened. Wings (Plate 1, fig. 21) pale yellow, the costal border slightly more saturated; small dark areas on r-m; both ends of basal section of vein M_3 but with the intermediate portion pale, m-cu; marginal spots at ends of all medial, cubital, and anal veins, largest and most conspicuous on 2d A. Venation: R_3 nearly transverse; cell 2d M_2 narrow to broadly sessile; vein 2d A strongly curved into margin but without angulation.

Abdomen obscure yellow, the caudal borders of the tergites narrowly darkened. Male hypopygium (Plate 3, fig. 46) with two broadly flattened spines on basistyle, *b*, these sessile or from very short lobes. Outer lobe of dististyle, *od*, with the basal portion pale and slightly dilated, with abundant setæ; more than the outer half of arm narrowed and darkened; no lobe at near midlength, as in *ensifera;* intermediate, *md*, and inner, *id*, arms of dististyle complex, especially the latter. Tenth tergite, *t*, with an elongate liguliform terminal lobe. Ninth sternite, 9*s*, broad, the usual modified outer setæ distinctly subterminal in position, the sternite projecting strongly beyond their insertion, narrowed and feebly darkened at apex.

Habitat.—Central Celebes (District Bontoe Batoe).

Holotype, male, Latimodjong Mountains, altitude, 3,800 feet, May 15, 1931 (*Clagg*). Allotopotype, female. Paratopotypes, 1 male, 2 females.

The other species of *Styringomyia* with bispinous basistyles (*armata* Edwards, *claggi* Alexander, *ensifera* Edwards) differ

conspicuously from the present fly in the structure of the male hypopygium. The latter comes closest to *ensifera*, differing in the conformation of both inner lobes of the dististyle and the lack of a medium lobule on the outer arm of the style.

STYRINGOMYIA SIBERIENSIS sp. nov. Plate 1, fig. 22; Plate 3, fig. 47.

General coloration of mesonotum pale, variegated with darker; scutellum black, with a yellow central spot; wings with brown clouds on r-m, m-cu, outer end of cell 1st M_2, and end of vein 2d A; vein 2d A angulated and more or less spurred near outer end; male hypopygium with the basistyle bearing a single lobe and spine; ninth sternite with two setæ.

Male.—Length, about 5.5 millimeters; wing, 4.2.

Female.—Length, about 5.3 millimeters; wing, 4.3.

Rostrum and palpi brownish black. Antennæ with scape and pedicel black; flagellum obscure yellow throughout. Head brownish yellow, sparsely pruinose; occipital region with a brown area on either side of the median line.

Pronotum pale medially, dark brown on sides. Mesonotal præscutum brownish gray, narrowly lined with darker brown; scutal lobes brown, the centers brownish yellow; median area of scutum yellow; scutellum black with a yellow median spot; mediotergite brownish black, sparsely pruinose; pleurotergite testaceous-yellow. Pleura variegated dark brown and obscure yellow. Halteres pale yellow throughout. Legs with the coxæ and trochanters yellow; remainder of legs yellow, the femora with two narrow dark brown rings that are interrupted beneath; tibiæ yellow, with two brown rings, the median one incomplete; tarsi yellow, the terminal segment abruptly darkened. Wings (Plate 1, fig. 22) yellow, with brown clouds on r-m, m-cu, outer end of cell 1st M_2, and end of vein 2d A; veins yellow, darkened in the clouded areas. Venation: Cell 2d M_2 narrow to more broadly sessile; vein 2d A angulated and short- to long-spurred at point of angulation.

Abdominal tergites more or less bicolorous, especially in male; obscure yellow in central portions, the bases and more narrow tips brown; sternites uniformly pale yellow. Male hypopygium (Plate 3, fig. 47) with the tenth tergite, *t*, produced into a conspicuous apical lobe; ninth sternite, 9*s*, slender, the two setæ close together. Basistyle, *b*, with the outer lobe slender, exceeding its terminal spine in length. Dististyle with the outer arm, *od*, provided with a series of four setæ at near midlength; inner arm, *id*, with the marginal spines in three groups; from near the

second of these groups arises a slender pale lobe that is tipped with a spine; discal setæ of inner arm about fourteen in number; intermediate arm, *md*, with apex produced into a single, powerful, black spine, with a comb of smaller black pegs just beneath it; a further comb of pegs some distance basad of the first group.

Habitat.—Eastern Siberia (Ussuri).

Holotype, male, Vinogradovka, 133° 50′ east longitude, 43° 20′ north latitude, August 10, 1929 (*Kiritchenko*). Allotopotype, female.

The types are preserved in the museum of the Russian Academy of Sciences.

As is usual in this involved genus, the present species is best defined by the structure of the dististyle of the male hypopygium, especially the armature of the intermediate and inner lobes. By Edwards's key to the species of *Styringomyia* [3] the present fly runs to *crassicosta* (Speiser), an African species. *Styringomyia siberiensis* is allied to two other species described at this time (*omeiensis* sp. nov. and *separata* sp. nov.), but differs from both in hypopygial characters. I had earlier [4] indicated the occurrence of this tropicopolitan genus in Siberia, this note being based upon the present record. The species is more northern in its distribution than any other so far made known, the most northerly previous record being for *S. nipponensis* Alexander, from Honshiu Island, Japan, latitude about 35° north.

STYRINGOMYIA OMEIENSIS sp. nov. Plate 1, fig. 23; Plate 3, fig. 48.

General coloration chiefly pale, the abdomen only slightly patterned; wings with the usual four dark spots; cell 2d M_2 broadly sessile; vein 2d A angulated and spurred at outer end; male hypopygium with the setæ of the ninth sternite slightly separated; dististyle with inner arm terminating in a single very long spine, with a group of about three smaller spines at its base.

Male.—Length, about 5 millimeters; wing, 4.8.

Rostrum brownish yellow; palpi light brown. Antennæ with the scape blackened beneath, obscure yellow above; pedicel brownish black; flagellum broken. Head chiefly pale.

Mesonotal præscutum pale, lined with brownish black on anterior portion; scutum chiefly pale; scutellum blackish, pale medially; mediotergite darkened. Pleura pale yellow. Halteres broken. Legs with the fore and middle coxæ pale yellow; femora

[3] Trans. Ent. Soc. London for 1914 (1914) 210–212.
[4] Philip. Journ. Sci. 52 (1933) 395.

pale yellow, each with two narrow dark rings that are slightly interrupted beneath; tibiæ with the apex and a premedial ring dark; tarsal segments whitish, the fore tarsi with the tips of the individual segments narrowly darkened; terminal segment black. Wings (Plate 1, fig. 23) pale yellow, with the usual four dark spots at r-m, m-cu, outer end of cell 1st M_2, and outer end of vein 2d A. Venation: Cell 2d M_2 broadly sessile, vein 2d A angulated and spurred at outer end.

Abdomen chiefly pale yellow, only slightly patterned at posterior borders of the tergites. Male hypopygium (Plate 3, fig. 48) with the ninth sternite, 9*s*, relatively broad at tip, the two setæ slightly separated at base. Armature of dististyle as shown; note that the apex of inner arm, *id*, terminates in one very long spine, surrounded at base by about three shorter ones; from this apical group the margin of the lobe slopes obliquely to base, with an almost continuous group of peglike spines along this margin; discal spines about a dozen in number.

Habitat.—China (Szechwan).

Holotype, male, Mount Omei, altitude 4,500 feet, August 9, 1929 (*Franck*).

Styringomyia omeiensis is most nearly allied to *S. separata* sp. nov. and *S. siberiensis* sp. nov., being most readily distinguished by the details of structure of the male hypopygium. As in the case of the species mentioned, by the use of Edwards's key to the species of the genus,[5] the present fly runs to *S. crassicosta* (Speiser), of Africa.

STYRINGOMYIA SEPARATA sp. nov. Plate 1, fig. 24; Plate 3, fig. 49.

Male.—Length, about 6 millimeters; wing, 4.5.

Female.—Length, about 5 millimeters; wing, 4.

Characters much as in *S. omeiensis* sp. nov., to which it is most nearly allied, differing especially in the structure of the male hypopygium.

Præscutum almost entirely brownish yellow, the dark markings best represented by four areas immediately before the suture and by the strongly darkened cephalic portion of the sclerite before the pseudosutural foveæ, the two regions interconnected by narrow darkenings on the interspaces. Wings (Plate 1, fig. 24) with vein 2d A very weakly angulated but unspurred. Male hypopygium (Plate 3, fig. 49) with the inner lobe, *id*, of

[5] Trans. Ent. Soc. London for 1914 (1914) 210–212.

the dististyle of distinctive conformation, the outer point terminating in about six powerful spines that are widely separated from the next group along the margin by a broad U-shaped notch; discal group of spines about ten in number, all long and slender; at base of inner lobe on mesal face with a small pale point.

Habitat.—Formosa (north).

Holotype, male, Rimozan, May 2, 1933 (*Issiki*).

Allotopotype, female, pinned with type.

ILLUSTRATIONS

[*a*, Ædeagus; *b*, basistyle; *d*, dististyle; *g*, gonapophysis; *i*, interbase; *id*, inner dististyle; *md*, intermediate lobe of dististyle; *od*, outer dististyle or outer lobe of dististyle; *p*, phallosome; *s*, sternite; *t*, tergite.]

PLATE 1

FIG. 1. *Trichocera arisanensis* sp. nov., venation.
2. *Longurio* (*Longurio*) *variceps* sp. nov., venation.
3. *Tipula* (*Acutipula*) *alboplagiata* sp. nov., venation.
4. *Tipula* (*Schummelia*) *indifferens* sp. nov., venation.
5. *Tipula* (*Schummelia*) *nigrocellula* sp. nov., venation.
6. *Tipula* (*Lunatipula*) *trialbosignata* sp. nov., venation.
7. *Tipula gressitti* sp. nov., venation.
8. *Nephrotoma atrolatera* sp. nov., venation.
9. *Dolichopeza* (*Nesopeza*) *lugubrivestis* sp. nov., venation.
10. *Limonia* (*Limonia*) *subhostilis* sp. nov., venation.
11. *Limonia* (*Dicranomyia*) *transfuga* sp. nov., venation.
12. *Dicranota* (*Rhaphidolabis*) *subsordida* sp. nov., venation.
13. *Dicranota* (*Rhaphidolabis*) *uninebulosa* sp. nov., venation.
14. *Dicranota* (*Rhaphidolabis*) *pallidithorax* sp. nov., venation.
15. *Dicranota* (*Amalopina*) *fumicostata* sp. nov., venation.
16. *Gonomyia* (*Gonomyia*) *justa* sp. nov., venation.
17. *Gonomyia* (*Lipophleps*) *toraja* sp. nov., venation.
18. *Gonomyia* (*Lipophleps*) *toala* sp. nov., venation.
19. *Lipsothrix kashmirica* sp. nov., venation.
20. *Ormosia hutchinsonæ* sp. nov., venation.
21. *Styringomyia celebesensis* sp. nov., venation.
22. *Styringomyia siberiensis* sp. nov., venation.
23. *Styringomyia omeiensis* sp. nov., venation.
24. *Styringomyia separata* sp. nov., venation.

PLATE 2

FIG. 25. *Tipula* (*Acutipula*) *alboplagiata* sp. nov., male hypopygium, details.
26. *Tipula* (*Acutipula*) *alboplagiata* sp. nov., male hypopygium, details.
27. *Tipula* (*Schummelia*) *indifferens* sp. nov., male hypopygium, details.
28. *Tipula* (*Schummelia*) *indifferens* sp. nov., male hypopygium, details.
29. *Tipula* (*Schummelia*) *nigrocellula* sp. nov., male hypopygium, ninth tergite.
30. *Tipula* (*Lunatipula*) *trialbosignata* sp. nov., male hypopygium, details.

FIG. 31. *Tipula* (*Lunatipula*) *trialbosignata* sp. nov., male hypopygium, ninth tergite.
32. *Tipula* (*Oreomyza*) *arisanensis* Edwards, male hypopygium, dorsal aspect.
33. *Tipula* (*Oreomyza*) *arisanensis* Edwards, male hypopygium, lateral aspect.
34. *Tipula gressitti* sp. nov., male hypopygium, details.
35. *Dolichopeza* (*Nesopeza*) *lugubrivestis* sp. nov., male hypopygium, details.

PLATE 3

FIG. 36. *Limonia* (*Limonia*) *subhostilis* sp. nov., male hypopygium.
37. *Dicranota* (*Rhaphidolabis*) *subsordida* sp. nov., male hypopygium.
38. *Dicranota* (*Rhaphidolabis*) *uninebulosa* sp. nov., male hypopygium, interbase.
39. *Dicranota* (*Rhaphidolabis*) *pallidithorax* sp. nov., male hypopygium, interbase.
40. *Dicranota* (*Amalopina*) *fumicostata* sp. nov., male hypopygium.
41. *Gonomyia* (*Gonomyia*) *justa* sp. nov., male hypopygium.
42. *Gonomyia* (*Lipophleps*) *toraja* sp. nov., male hypopygium.
43. *Gonomyia* (*Lipophleps*) *toala* sp. nov., male hypopygium.
44. *Lipsothrix kashmirica* sp. nov., male hypopygium.
45. *Ormosia hutchinsonæ* sp. nov., male hypopygium.
46. *Styringomyia celebesensis* sp. nov., male hypopygium.
47. *Styringomyia siberiensis* sp. nov., male hypopygium.
48. *Styringomyia omeiensis* sp. nov., male hypopygium.
49. *Styringomyia separata* sp. nov., male hypopygium.

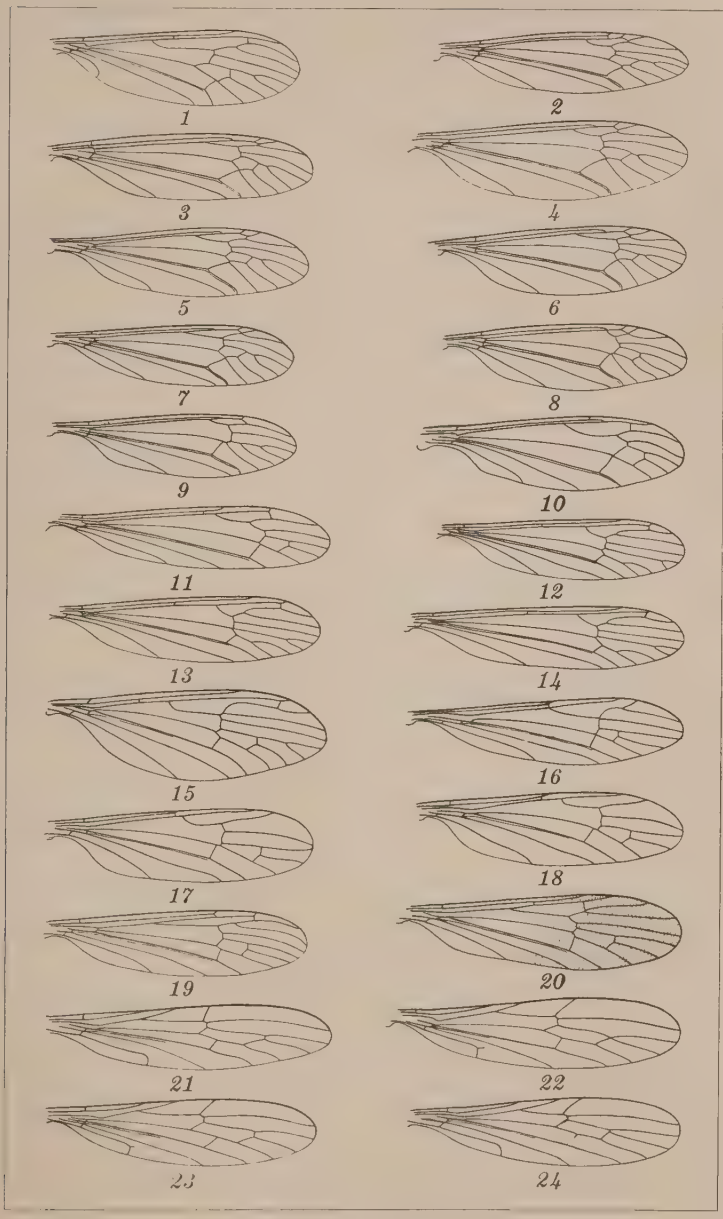

PLATE 1.

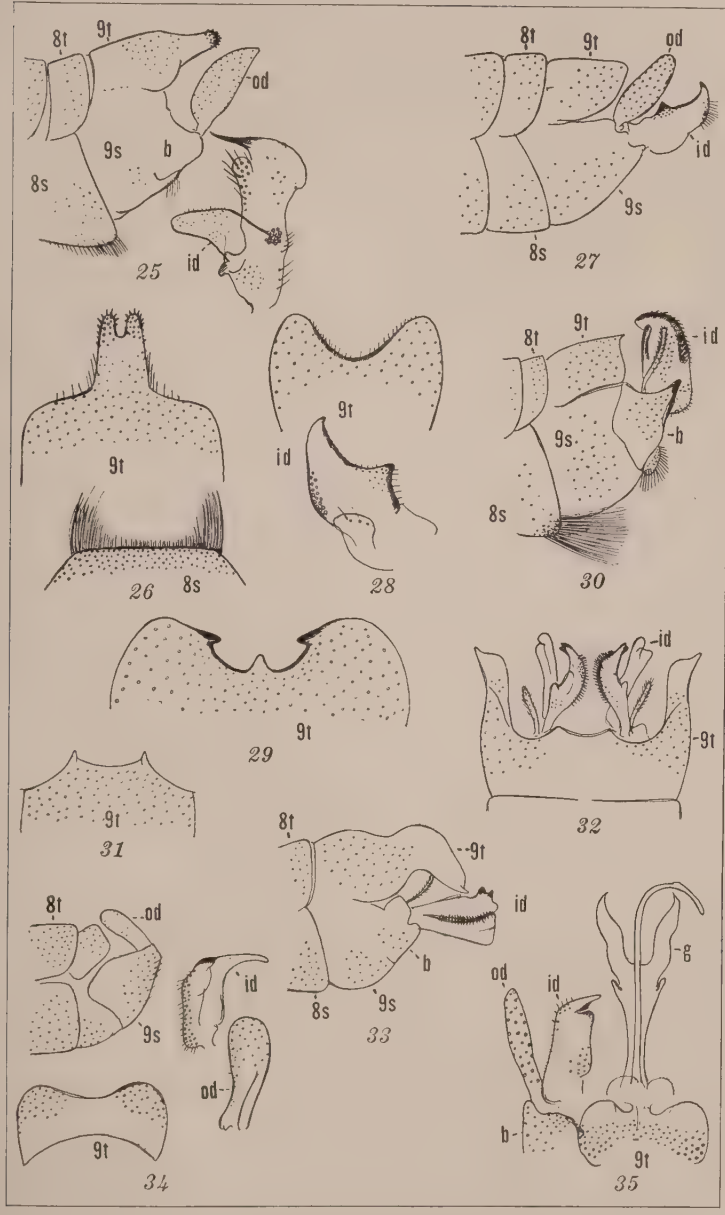
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PLATE 2.

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9s
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od
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id
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PLATE 3.

SPERMATID TRANSFORMATION IN AMBLYCORYPHA OBLONGIFOLIA (DE GEER), A TETTIGONIID

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TWO PLATES

INTRODUCTION

This study gives a detailed account of the metamorphosis of the spermatids in *Amblycorypha oblongifolia* (De Geer), a tettigoniid, and follows the changes taking place during the process.

While cytologists have worked on the spermatocyte divisions of many species of Tettigoniidæ, only a few have published papers dealing with spermatid transformation. Sabatier (1890), Otte (1907), and Davis (1908) are among the few investigators who have worked on spermatid transformation. The accounts of Sabatier and Davis, however, are rather short and show an incomplete series of changes. The paper of Otte, as far as we know, is the only one that gives a detailed history.

MATERIAL AND METHODS

The species selected for this study is *Amblycorypha oblongifolia*. Its spermatogenesis has not been worked out before. All of the material was obtained from adult individuals, collected in the neighborhood of the University of Kansas during July and August, 1932.

Various fixing agents were tried, but Flemming's fluid and Benda's proved by far the best. The material was stained in sections 6 and 10 microns thick. Many staining combinations were tried, but Benda's method and Heidenhain's iron-hæmatoxylin with a counterstain gave the most satisfactory results.

Observations were made on both sectioned and live material, but mostly on the former. The "intravitam technic," developed by Baumgartner and Payne (1931), was used whenever observations on live cells in the follicles were made. Smear preparations were also used for mature spermatozoa.

OBSERVATIONS

THE TESTES

Amblycorypha oblongifolia, one of the "false katydids," has paired testes. Each testis lies in the dorsal side of the abdomen, extending from the third to the sixth abdominal segment. In the adult the testis looks like a yellowish, somewhat flattened, strawberry.

Each testis is composed of many short cylindrical follicles of varying length. The follicles, which lie nearly parallel to each other, are usually larger in the middle and somewhat tapering at either end, depending upon the age of the individual, and are inclosed in a thin connective tissue containing yellowish pigment. Every follicle is made up of many cysts, which vary in size. In follicles of young individuals sectioned longitudinally, the germ cells are generally arranged in successively older generations, the spermatogonia lying at the blind end of the follicle, followed by the spermatocytes, which in turn are oriented in such a way that their heads point toward the blind end of the follicle.

SPERMATID FORMATION

As a result of the two spermatocyte divisions, four spermatids are produced from each primary spermatocyte. Two of these spermatids differ from the other two only in the possession of a large nuclear element—the compact accessory chromosome—otherwise they are structurally the same. Plate 1, figs. 9 and 10, represents two young spermatids, one having the extra element and the other lacking it. Figure 14 shows a spermatid at a little later stage. It shows the fully formed nucleus and the nebenkern. Figure 16 shows the acroblast. The nucleus, nebenkern, and acroblast are three of the most common and characteristic structures of most orthopteran spermatids that have been studied. Before proceeding to describe in detail the changes undergone by these structures during the process of spermatid transformation, the history of their origin will be traced.

The nucleus.—The spermatid nucleus pictured in figs. 14 and 15 arises from the anaphase chromosome plates of the second maturation division (figs. 2 and 3). During the anaphases, there is nothing of particular interest in the behavior of the chromosomes of this tettigoniid. They are distributed in the usual manner, one-half migrating to one pole of the spindle and the other half to the other pole (fig. 1), where they aggregate

at a little later stage (fig. 2). At this time the outlines of the individual chromosomes are still distinctly visible, but as the chromosomes fuse into a compact mass, all trace of their outlines is lost to view, except that of the accessory chromosome, which maintains its identity. Figures 3 and 6 show two cells in which the accessory chromosome is protruding from each compact mass of chromosomes (autosomes) and extending into the cytoplasm. In the telophases this compact mass resulting from the fusion of the chromosomes begins to show signs of internal disintegration (fig. 8). When the two daughter cells separate, the process of disintegration of the chromosome mass is well under way, the vacuoles in each having greatly increased in number, and every vacuole having become larger and more distinct (fig. 9). From this stage the mass of chromatin rapidly breaks up into patches of varying size, which in turn disintegrate into smaller pieces and larger granules. This breaking up of the mass gradually increases the size of the nucleus, which gains in volume at the expense of its density, while at the same time the substance itself becomes less chromophilic (figs. 10 to 13). When the nucleus reaches its maximum size (fig. 14), the nuclear membrane appears and the spermatid nucleus is completed. A glance at figs. 9 and 14 shows the increase in size of the nucleus from the time of separation of the two daughter cells until the formation of the nuclear membrane. These illustrations also show the gradual loss of affinity for nuclear stain.

The accessory chromosome, whenever present, remains intact throughout the stages of the formation of the spermatid nucleus. Its behavior differs markedly from that of the autosomes, in that it has not undergone a process of disintegration. It retains its compact form, and its staining reaction is similar to that of autosomes in metaphase. Before the formation of the nuclear membrane, it is usually found lying close to the periphery of the chromosome mass (figs. 10 to 13). After the appearance of this membrane, it takes a position close against its inner surface. It usually assumes an oval or lenticular shape. Because of its large size and staining reaction, it is easily distinguished from the rest of the nuclear elements, and appears very conspicuous in young spermatids.

The nebenkern.—The second outstanding characteristic structure of the spermatid is the nebenkern. In the early anaphase of the second maturation division, the mitochondria are found in the cytoplasm in the form of short rods and large granules.

They are always closely crowded together midway between the poles, just under the cell wall (fig. 1). In the middle anaphase the granular mitochondria have increased in size and become short rods of varying length. At this stage they are all in the form of short rods (fig. 2). They are closer to the spindle plate due to the elongation of the cytoplasm. In the next stage (fig. 3) many of the rods, especially those lying nearest the cell wall, join end to end to form long threads. These threads at first show the outlines of the short rods from which they are formed. At this stage a cross section of the cytoplasm below the chromosome plate (fig. 4) reveals the unequal distribution of the mitochondria around the spindle. At a little later stage (figs. 5 and 6) many of the short rods, by the process already described, become long threads, which extend from pole to pole of the spindle. A polar view of the cytoplasm of a cell in the same stage, as indicated in fig. 6, shows almost uniform distribution of the mitochondria (fig. 7). It is at this time that the chromosome plates begin their telokinetic movements, and the spindle fibers begin to disappear. In late telophase (fig. 8) the number of the long mitochondrial threads has increased greatly, but the number of the short rods has decreased. The long threads apparently become homogeneous in structure, for they stain uniformly. When the two daughter cells finally separate, the long threads, which extend from pole to pole, are divided transversely into approximately equal parts. The short rods, which lie at the interzonal region, are also divided. The rest of the short rods have been previously assorted to the cytoplasm on the polar side of each nucleus. Hence, the separation of the two daughter cells also divides these meristically, with the result that approximately one-half of the entire mitochondrial substance is assorted to each of the resulting spermatids.

Later the mitochondria in each spermatid clump into a rather loose mass near the nucleus, which is always found to lie on that side of the spermatid where the cytoplasm is greatest and almost diametrically opposite the centriole (fig. 9). This mass rapidly shortens and condenses, first assuming a more or less rectangular form, which stains darker at both ends (fig. 10). At this stage its threadlike structure is still distinct, but disappears as it rounds out into a ball-shaped body (fig. 14). This spherical body, which stains homogeneously with basic stains, is the so-called nebenkern.

The acroblast.—The third spermatid structure is the acroblast, which differentiates first as a small vesicle from the fused mass

of dictyosomes lying against the nuclear membrane. During the anaphases of the second spermatocyte division, the dictyosomes are distributed in the cytoplasm. Most of these lie outside the spindle plate in the early anaphase (fig. 1). At a little later stage (fig. 2), some have invaded the spindle plate. In the late anaphase (fig. 3), the greater number of them arrange themselves along with the mitochondrial threads. Later, apparently one-half of them move toward the clear space on the equatorial side of each chromosome plate (figs. 5, 6, and 8). When the two daughter cells separate, each carries with it a certain number of dictyosomes. The dictyosomes assorted to each cell migrate close to the periphery of the nucleus (fig. 9), where they fuse to form a comparatively large darkly staining body (figs. 10 to 13). At first this mass of dictyosomes is not applied to the nucleus; but later, as the nucleus increases in size, it gradually comes in contact with it (figs. 12 and 13). When the nuclear membrane appears, the mass of dictyosomes is found lying against its outer surface with a small vesicle already differentiating from it (fig. 14). Soon this vesicle becomes enlarged and more evident (figs. 15 and 16). It is the spermatid acroblast.

The structure of individual dictyosomes may be distinguished in many of the stages described. They consist of a darkly staining or chromophilic part and a nonstaining or chromophobic part. The chromophilic part has the shape of a crescent, without any visible horns extending from the ends. It only partially incloses the chromophobic part (figs. 1 to 4). The number of dictyosomes found in the second maturation division varies from six to twelve, but the most probable number is the latter, as that is the one most frequently met with in many of the stages described.

All of the stages described above are of short duration. Throughout the formation of the nebenkern the centriole, always surrounded by a hyaloplasmic area, is invariably found to be approximately opposite that of the forming nebenkern.

SPERMATID TRANSFORMATION

The stage shown in fig. 14 may be taken as representing the completion of the spermatid as a cell and the beginning of the transformation of the spermatid into the mature spermatozoön.

The nucleus.—The nucleus as previously described has reached its maximum size at this stage. It measures approximately twice the diameter of the nucleus at the time of the separation of

the two daughter cells. From this stage the nucleus slowly decreases in size until it reaches the stage just before it elongates (fig. 33). The chromatin patches continue to disintegrate into smaller masses, which in turn break down into small pieces and granules (figs. 15 to 18). Later these chromatin pieces and granules are distributed just beneath the nuclear membrane, leaving the center of the nucleus almost clear (figs. 19 to 21). Shortly after this, chromatin threads invade the clear space, and in many spermatids large chromatin patches of varying size also make their appearance. These large patches of chromatin are the products of the disintegration of the accessory chromosome. Figures 18 and 21 show two spermatids in which the accessory chromosome begins to disintegrate. The remnants of the accessory chromosome are connected with one another by chromatin threads. Many of these threads extend from the periphery of the nucleus, where the concentration of chromatin granules is greatest, into the central area (figs. 22 to 30). Later the remnants of the accessory chromosome rapidly break down into small pieces, while the central vacuoles round out. Very often a large chromatin granule, which is a persisting remnant of the accessory chromosome, is found in the center of many of them (figs. 32 to 34). The nucleus reaches its minimum spherical size just before it elongates (figs. 32 and 33). Then it begins to elongate (figs. 34 and 35), and flattens out rapidly like a paddle (figs. 36 to 38). During these processes of elongation and flattening, the nucleus loses its symmetry, for one side elongates and flattens out faster than the other, which bulges out instead. During this stage of development the chromatin granules continually shift their position until they finally form a ring in the center of the nucleus. This ring gradually becomes flattened and elliptical. It is surrounded by the nonstaining or oxyphilic part of the nucleus, which forms the central clear space of the nucleus in the earlier stages of the transformation. Figures 35 to 38 will give a much better idea of the changes in the nucleus than can possibly be conveyed in a detailed description. The elongation of the nucleus proceeds, while at the same time the granular structure of its basichromatin part disappears, with the exception of a small portion at the anterior end, which still remains granular (figs. 39, 43, and 44). Soon the entire basichromatin part becomes more and more compact and finally becomes homogeneous and stains very intensely in the last stages of the transformation. No elimination of a nuclear substance into the cytoplasm has been observed during the whole process.

The final product of the transformation of the spermatid nucleus is the solid deeply staining fusiform sperm nucleus (fig. 47). Figure 48 represents a cross section of two sperm nuclei.

The nebenkern.—After the mitochondria have aggregated into an apparently homogeneous mass (fig. 14), a rapid process of vacuolization takes place. A few vacuoles appear at first just beneath the periphery. Each is separated from the others by a ring of chromophilic substance (fig. 16). Then the vacuoles coalesce to form a few larger vacuoles, which are separated one from the other by a chromophilic septum. The septa radiate from a darkly staining center to the outer ring (fig. 17). These septa soon disappear, and a condition like that shown in fig. 18 results. The nebenkern now consists of a chromophilic center, surrounded by a chromophobic part. Very often small vacuoles are found in the chromophilic central substance. The stage shown in figs. 18 and 19 persists for a long time and may be called the resting stage of the nebenkern. Following this it elongates and approaches the axial filament until it comes to lie parallel with it. Then its central core vacuolizes (fig. 20) and continues to elongate. Later it divides, one-half sheathing one side of the axial filament and the other half the other side (fig. 21). At first large vacuoles are found in its component parts; but, as it elongates more and more, it breaks down into smaller pieces and the vacuoles disappear (figs. 25 to 27). The elongation seems to be associated with the lengthening of the axial filament, for, as the latter structure increases in length, the nebenkern trails it (figs. 29 and 32). In the later stages of the nebenkern, "blebs" appear in it, which are pushed down as the spermatozoön is about to reach its final development, and form a large protoplasmic ball at the posterior end of the flagellum (fig. 44). This protoplasmic ball is finally cast off as the spermatozoön becomes mature. The final product in the metamorphosis of the nebenkern is a sheathlike structure, which forms the outer covering of the flagellum of the mature spermatozoön (fig. 47).

The acroblast and the acrosome.—The acroblast arises, as we have said, as a small vesicle differentiated from the dark mass of dictyosomes lying against the nuclear membrane near the nebenkern. This structure rapidly increases in size and assumes a rounded shape. It stains heavier on the side close to the cell wall, and is always in contact with the dictyosome mass from which it is differentiated (fig. 16). It then begins to migrate

around the nucleus. During the process of migration it gradually increases in size (figs. 17 to 23). After its migration around the nucleus, it stops at its original position and remains stationary for a long time, and again increases in size. It appears to stain very lightly at this stage (figs. 24 and 25). Later the acroblast rapidly separates from the rest of the dictyosome mass, which has become diffused now, and disappears in the cytoplasm. Figures 26 to 28 show three stages in the separation of the vesicular acroblast (acroblast remnant) from the diffused mass of dictyosomes. Figure 29 shows the remnant of the acroblast passing down the tail region. Meanwhile the rest of the dictyosome mass, which lies against the nuclear membrane, becomes diffused and loses its strong affinity for nuclear stains as it increases in size (fig. 25). Later it becomes distinctly demarcated from the separating acroblast, and rounds out (figs. 26 and 27). At this time vacuoles appear in it. These vacuoles coalesce to form larger vacuoles, which increase in size and produce a large vesicle in the center of which is a darkly staining granule (fig. 28). The vesicle and the granule found in it form the acrosome complex. The vesicle and the granule rapidly increase in size as they move toward the apex of the nucleus, opposite the centrioles (figs. 29 and 30). After the acrosome complex has reached the point opposite the centrioles, the cell's axis straightens. Thus, the centrioles become definitely located at the posterior end of the nucleus, and the acrosome complex at the anterior (fig. 32). The acrosome complex soon flattens out against the nucleus. Meanwhile the granule of the complex augments in size, while the clear vesicle begins to show distinct affinity for basic stains (fig. 33). As the nucleus elongates and flattens out, the acrosome complex, which now stains homogeneously, extends for some distance around its anterior end, forming an intensely staining caplike structure (figs. 34 to 38). Later the caplike structure (acrosome) becomes horseshoe-shaped (fig. 39). Then it assumes the shape shown in fig. 40. At a little later stage (fig. 43) a marked change occurs in it. It no longer stains uniformly, its median portion staining only slightly, while the part on either side plus a small anterior portion remain deeply stained. However, this difference in staining reaction disappears when the acrosome passes into the last stages of its transformation (figs. 44 and 47), and the spermatid becomes a mature spermatozoön. As shown in figs. 44 and 47, the acrosome loses most of its strong affinity for stain. It is only slightly chromophilic now. Its final defin-

itive shape is in the form of an A without the bar or like an inverted V.

The centrioles and the axial filament.—After the separation of the two daughter cells in the last maturation division, the spheroidal centriole, surrounded by a hyaloplasmic area, appears on that side of the spermatid opposite the chondriosome mass (fig. 9). As the chondriosome mass condenses and rounds out, the centriole maintains its position in relation to this structure; that is, it is always found approximately opposite the chondriosome mass (figs. 10 to 13). After the nebenkern has been formed, the centriole, now in contact with the nucleus, begins to move toward it (fig. 14). At this stage it is hard to tell whether or not the centriole has divided, because it is closely associated with a darkly staining mass of nuclear substance, which lies just beneath the nuclear membrane (figs. 16 and 17). In later stages, however, the centriole is seen already divided into proximal and distal parts, apparently equal in size. There is a very fine granule, scarcely thicker than the axial filament on which it is lying, a short distance from the distal centriole. This may be called the distal centriole derivative (figs. 18 and 19). After the nebenkern has reached the axial filament, it begins to elongate. Then the distal centriole derivative moves caudad and comes in contact with the elongating nebenkern at the posterior end (figs. 20 and 21). The centriole derivative continues to move posteriorly and is generally found at the end of the rapidly elongating nebenkern (figs. 25 and 32) up to a very late stage of the spermatid transformation when it is lost to view and its subsequent fate becomes unknown. At first the proximal and distal centrioles are nearly of the same size, but in later stages the former becomes larger than the latter (figs. 20 to 22, 27, 28, 32, and 33). When the nucleus begins to elongate, the centrioles become indistinct and are closely associated with, perhaps are, the same substance previously described in the earlier stages. This substance forms an envelope around them (figs. 34 and 35). As the nucleus becomes paddle-shaped and the break in the nuclear membrane at the posterior end becomes more evident, each centriole appears to have divided longitudinally into two (figs. 36 to 38). The position of the centrioles in relation to the basichromatin part of the nucleus as shown in fig. 36 lasts for a long time. They do not come in contact with it, but are separated by a space which is filled with very fine granules (figs. 37 to 43). In later stages the centrioles fuse, and with

the enveloping substance form a small compact mass (fig. 43). When the nucleus finally becomes solid and stains intensely, the fused centrioles come in contact with it at the posterior end to form the middle piece of the mature spermatozoön (figs. 44 and 47).

The spermatozoön.—The most mature spermatozoa found in our preparations made in the early part of summer have the following parts:

1. A large slightly staining acrosome, which resembles an inverted V. It forms the most anterior end of the spermatozoön.
2. A solid fusiform nucleus, which stains intensely with basic stains. There is no visible membrane surrounding it.
3. A small compact middle piece connected anteriorly with the nucleus and posteriorly with the flagellum.
4. A comparatively short flagellum surrounded by a delicate sheath. Its length is approximately twice that of the head.

DISCUSSION

The metamorphosis of the spermatid into the spermatozoön in *Amblycorypha* shows remarkable changes and offers many points of interest. These changes resemble in a general way those described by Sabatier (1890) in several species of Tettigoniidæ, by Otte (1907) in *Locusta*, and by Davis (1908) in *Steiroxys*. In many details, however, it differs widely from the changes described by these authors. These differences, we are inclined to believe, are due mostly to differences of interpretation.

THE NUCLEUS

The transformation of the spermatid nucleus into the sperm nucleus in *Amblycorypha* offers but one interesting point that is worth discussing. This is the differentiation of the nuclear substance into two distinct substances, one of which shows a strong affinity for basic stains, the other little or none at all. The former substance is called basichromatin and the latter oxychromatin. This differentiation, which occurs before the condensation of the nucleus to form the compact deeply staining sperm nucleus, seems to be of widespread occurrence in Tettigoniidæ. It has been partly described by Otte (1907) and by Davis (1908). The same differentiation has been described by Montgomery (1911) in *Euschistus*, by Bowen (1920) in some species of Hemiptera, and by others in other forms.

Now and again a remarkable phenomenon in the transformation of the spermatid nucleus has been reported by several investigators of insect spermatogenesis. This phenomenon is the extrusion into the cytoplasm of a lightly staining substance. Montgomery (1911) and Bowen (1922) have observed and described it. This interesting phenomenon, while perhaps of widespread occurrence in Hemiptera, does not seem to occur in Orthoptera. At least, it has not been reported in the spermiogenesis of many orthopterans thus far examined; and as far as our observations on *Amblycorypha* are concerned, there is nothing to indicate that such a phenomenon occurs in the metamorphosis of the spermatid in this insect.

THE NEBENKERN

The extensive literature on the origin and behavior of this structure has been so thoroughly reviewed by recent investigators, notably Bowen (1922), that it will not be necessary to present a detailed discussion here.

In general there are two opinions held concerning the origin of this characteristic structure in the spermatids of insects. One view maintains that the nebenkern arises from the fusion of the chondriosomes assorted to each spermatid; the other, that it originates from the direct metamorphosis of the interzonal filaments. The former view is supported by the works of such prominent investigators as Otte (1907), on *Locusta;* Montgomery (1911), on *Euschistus;* Payne (1917), on *Gryllotalpa;* and recently by the works of Bowen (1922), on several species of Hemiptera-Heteroptera; Pollister (1930), on *Gerris;* Johnson (1931), on *Oecanthus;* and M. A. Payne (1933), on several species of grasshoppers. The latter view is supported by the researches of Butschli (1871), on several species of insects; La Vallette St. George (1886), on *Blatta;* Paulmier (1899), on *Anasa;* and Munson (1906), on *Papilio.*

Our observations are in line with the view held by the first group of investigators. The nebenkern in this tettigoniid is derived entirely from the fusion and subsequent condensation of the chondriosomes.

Sabatier (1890) seems to be one of the first to observe the nebenkern in the tettigoniid spermatid. He believes this structure arises from the protoplasm (cytoplasm) and calls it "vesicule protoplasmique." What finally becomes of this vesicle, he

does not say. Since in his brief paper he neither gives figures nor describes the changes following the formation of this structure, it is impossible to compare the behavior of his "vesicule protoplasmique" with the behavior of the nebenkern in *Amblycorypha.*

Otte (1907) describes in detail the origin and behavior of the nebenkern in *Locusta.* According to his account, the greater portion of the mitochondria assorted to each spermatid fuses into a loose mass near the side of the interzonal filaments. This mass of mitochondria becomes condensed and forms the compact nebenkern, which stains darker than the cytoplasm. Later the nebenkern differentiates into two substances; namely, an inner chromophilic core and an outer chromophobic part. From the chromophilic center septa radiate and extend to the periphery of the chromophobic part. Then the nebenkern becomes homogeneous and finally elongates to form a sheath around the axial filament.

Davis (1908) fails to identify the nebenkern in the spermatids of *Steiroxys,* nor does he trace the origin of a structure that corresponds to it. He calls "Nebenkern" for convenience and "without implying anything in regard to its homologies," a darkly staining structure which lies near the nucleus (see his fig. 203.) If his "Nebenkern" corresponds to the acroblast of recent investigators, then the less deeply staining structure applied to the nuclear membrane and lying near the "Nebenkern" is probably the true nebenkern. However, if we were to interpret his figure 203, with reference to the differential staining of the two structures and their relative position in the cytoplasm, we would say the nebenkern is the same one which he calls "Nebenkern" (acroblast), and the less deeply staining body closely applied to the nuclear membrane is the acroblast.

Our observations on the origin and behavior of the nebenkern in *Amblycorypha* agree in general and in many particulars with those of Otte in *Locusta.* However, he has not mentioned a few of the early stages in the differentiation of the nebenkern into two substances. In these particulars our account resembles closely the description given by Bowen (1922), of *Murgantia,* and partly that given by Johnson (1931), of *Oecanthus.* The differentiation of the nebenkern in some hemipters, as given by Bowen, with the exception of the initial stage—namely, the appearance of many vacuoles in the periphery of the nebenkern—

is so strikingly like that of *Amblycorypha* that it could be applied almost directly. Bowen states that—

> . . . once fully completed, the nebenkern begins to show the first signs of differentiation into two substances. This first makes itself evident by the appearance of a large number of vacuoles in the periphery of the mitochondrial mass. These are at first very indistinct and difficult to demonstrate, but they presently become clearer and form a complete layer investing the periphery of the nebenkern. They seem to stain little or not at all in contradistinction to the central mass, which is more darkly colored. This may be due to the fact that the process of differentiation is going on from without inward, or it may be due to the optical superposition of several layers of vacuoles. The outermost layer clears up very rapidly, the separate vacuoles fusing together to form large clear spaces, separated from each other by septa which pass outward from the central mass to the exterior of the nebenkern which is marked by a very definite membrane. The whole thing reminds one of the figure given by Meves (1900) of Pygaera. The clearing up of the outer layer of vacuoles now proceeds rapidly, the substance of the vacuole walls apparently withdrawn into the underlying region of the nebenkern, and eventually the whole peripheral zone appears as a clear non-staining envelope, enclosing a central chromophilic core.

While the processes of the differentiation of the nebenkern into a chromophilic central substance and a chromophobic outer substance in *Amblycorypha* resemble more closely those in Hemiptera than those in *Locusta* as described by Otte, the subsequent changes in *Amblycorypha* are entirely different from those observed by Bowen and Johnson, but are similar to those described by Otte. As a whole the changes undergone by the nebenkern in *Amblycorypha,* from the time of its formation to its final fate, resemble more closely those of *Locusta* than those described either by Bowen in Hemiptera or by Johnson in *Oecanthus.* This resemblance is not surprising at all—in fact, it is what one would expect to find in such closely related genera as *Locusta* and *Amblycorypha.* It appears very probable that, if a more-detailed study of the nebenkern in *Locusta,* in the forms examined by Sabatier and in *Steiroxys* studied by Davis, were made, a condition similar to that in *Amblycorypha* would be revealed.

THE ACROSOME

No structure in the spermatids of insects has received more consideration in the past than that which forms the apex of the sperm head, commonly called the acrosome or perforatorium. This structure, which varies greatly in size and shape in diverse

groups of animals, has been traced to many different origins. In insects alone it has been described as originating from the nucleus, spindle remnants, nebenkern, Golgi bodies, idiosome, acroblast, sphere, from a combination of mitochondria and central spindle fibers, from a centrosome, and in some cases it has been derived from an obscure structure in the cytoplasm, the nature of which is not known.

Butschli (1871) appears to be the first to describe the formation of the acrosome in the tettigoniid spermatids. He derives this structure from a granulelike vesicle in the cytoplasm. This vacuole comes in contact with the nucleus and moves forward to its anterior portion to form the definitive acrosome. His observations are in line with those of many recent investigators. He fails, however, to make out the origin of this granulelike vesicle and to notice the casting off of a substance which corresponds to the so-called acroblast remnant.

Sabatier (1890) in his studies of the spermatogenesis of some locustids traces the origin of the acrosome to the "vesicules nucleaires," which arise from the direct metamorphosis of the nucleus. A fusion occurs among these small nuclear vesicles, and as a result a small number of large vesicles is produced. These large vesicles increase in size, lose gradually their affinity for stain, and diminish by a further fusion to three; namely, "un mediane, petite et saillante, et deux laterales qui s'allongent et finissent par former les deux branches ou crochets de l'ancre que constituent la coiffe cephalique."

It seems probable that Sabatier misinterprets the complicated changes occurring during the transformation of the spermatid nucleus in the locustid forms he has examined. His observations on the origin of the acrosome are entirely different from our observations on *Amblycorypha* and from those of others who have later made careful studies on the origin of this structure in closely related insects.

Otte (1907) gives a complete, as well as a most satisfactory, account of the formation of the acrosome in *Locusta*. Our account of the origin of the acrosome in *Amblycorypha*, with the exception of two important points, is in accord with his on *Locusta*. In his observations on the origin of the idiosome, the structure in the spermatid from which the acrosome originates, he describes it as arising from the direct metamorphosis of the bundle of central spindle fibers (interzonal filaments) and a portion of the mitochondria. As far as our observations are

concerned, we are quite sure that the central spindle fibers and a portion of mitochondria take no part in the formation of the acroblast in *Amblycorypha.* Here the central spindle fibers disappear after the telokinetic movements of the chromosome plates have taken place; while the acroblast makes its appearance only after it has been differentiated from the fused mass of dictyosomes lying against the nuclear membrane, long after the disappearance of the spindle fibers and the aggregation of the mitochondria into a compact spherical body. Recent investigations on this subject show conclusively that the idiosome or acroblast does not arise from the spindle fibers and mitochondria but from other structures in the cell, such as Golgi bodies and dictyosomes.

Again Otte describes the casting off of the mitochondrial substance from the idiosome after its rotation around the nucleus. In *Amblycorypha* the substance that is cast off from the forming acrosome is the rounded vesicle (acroblast remnant), which corresponds to his idiosome. In short the idiosome itself forms the acrosome in *Locusta,* while in *Amblycorypha* the acrosome is formed by the acrosome complex synthesized in close connection with the acroblast.

Davis (1908) describes the acrosome in *Steiroxys* as arising from a rounded structure which he calls, for convenience, "Nebenkern." The nature of this body he does not know. He traces the transformation of this structure into the anchor-shaped acrosome. He has not observed, however, the differentiation of the acrosome complex in close association with the "Nebenkern," and the casting off of a substance from it. He also overlooks some of the later stages of the acrosome.

Recently the origin of the acroblast and its rôle in the formation of the acrosome have been more fully studied in other insects that are not closely related to *Amblycorypha.* A brief review of the results of a few of these investigations will be given here for comparison with our findings.

Payne (1916), in his study of the germ cells of *Gryllotalpa,* like Davis, is not able to ascertain the origin of the acroblast. He observes two structures in the cytoplasm of older spermatids, one of which is elongated and curved and is in contact with the nuclear membrane, the other small and spherical. The elongated body gives rise to the acrosome, while the spherical one degenerates and disappears during the transformation. Concerning the origin of these structures, Payne says, "it would seem that

they rise de novo in the cytoplasm as they are not present in the young spermatids."

Bowen (1920 and 1922), in his study of the spermatogenesis of many hemipters describes the fused type of acroblast as originating from the fusion of the dictyosomes assorted to each spermatid. From the acroblast the acrosome complex is differentiated, after which the acroblast remnant is cast off from the acrosome complex. This complex, which consists of the acrosomic vesicle and the deeply staining granule found in it, gives rise to the definitive acrosome. This differentiation of the acrosome from the acroblast seems to be of very wide application in insects. It has been found in many of the insects thus far examined in detail.

Voinov (1925) describes the formation of the acrosome in *Gryllotalpa vulgaris*. It arises, according to him, from two structures; namely, the acroblast and the "appareil spherulaire." The acroblast arises in close association with the Golgi bodies, while the "appareil spherulaire" arises by the fusion of spherical bodies in the spermatid. This process of acrosome formation is slightly different from the condition observed by Bowen. However, both involve the acroblast as participating in the formation of the acrosome.

F. Payne (1927) in another paper finds that the acrosome originates from a large idiosome. According to his account, several small spheres, which he calls proidiosomal spheres, fuse to form a large idiosome, around which the Golgi bodies collect or aggregate. This complex corresponds to the acroblast of other investigators. However, in *Gelastocoris* the large idiosome alone is responsible for the formation of the acrosome, the Golgi bodies being cast off. Thus, while the acrosome is the product of the acroblast in the forms studied by Bowen, in *Gelastocoris* it is the product of the large idiosome. In both cases a substance is cast off from the structure that finally forms the definitive acrosome.

In *Gerris*, Pollister (1930) traces the origin of the acrosome from the acroblast. He observes that the osmiophilic Golgi materials aggregate in the young spermatid and form "an irregularly folded, sac-like structure," the acroblast. After the formation of the acrosome, the acroblast remnant separates from it and is cast off into the cytoplasm.

Johnson (1931) finds in *Oecanthus* a condition nearly like that described by Bowen. The acroblast is produced by the fusion in the spermatids of a number of dictyosomes, from which the

acrosome is apparently differentiated. Later the acroblast remnant is cast off into the cytoplasm and passes down the tail.

Our study of the results of the above investigators and many others reveals that in many cases the acrosome in insects is formed in close association with the acroblast or a structure similar to it.

THE CENTRIOLES AND THE AXIAL FILAMENT

The behavior of the centrioles during the transformation of the spermatid is so different in various groups of animals that only observations on closely related genera will be considered in this brief discussion. Those desiring a lengthy discussion on this particular subject are referred to the works of Bowen (1922), Baumgartner (1929), and Johnson (1931).

Early workers on the spermiogenesis of the tettigoniids have failed to demonstrate the presence of centrioles in the spermatids. Perhaps this is not because centrioles are not present in the objects examined, but because they are overlooked or considered insignificant or because the technic used at the time is not adequate to bring out such minute structures.

Otte (1907) seems to be the first to demonstrate the centrioles in the locustid spermatids. He traces the behavior of these bodies throughout the transformation of the spermatid. The centrioles, according to his account, are so small that they are not easily recognized. He finds two small granules near the periphery of the cell, which he assumes to be central bodies. Later these are oriented in proximal and distal positions, the larger proximal centriole lies at the nuclear membrane and the distal one at the cell wall. Between them is the intracellular axial filament and extending from the distal centriole is the extracellular axial filament. The proximal centriole is usually so closely associated with the accessory chromosome or with chromatin patches that it cannot be recognized. Later this association becomes so close that it gives one the impression, "als käme der Achsenfaden von dem akzessorischen Chromosom." When the accessory chromosome breaks down, the proximal centriole emerges and then divides laterally into two. Each of the later divides, giving rise to four centrioles. The two inner centrioles move up into the nucleus, and each again divides into two. These four centrioles resulting from the division of the two, migrate farther to the anterior pole of the nucleus and form an "Innenkörper" in the sperm nucleus. Meanwhile the

two centrioles left at the nuclear membrane elongate and take part in the formation of the middle piece. The distal centriole continues to move caudad until it disappears from view in the last few stages of the transformation.

The behavior of the centrioles in *Amblycorypha* differs greatly from that described by Otte for *Locusta.* In both genera the centrioles are similar. In both chromatin material is in close association with the centrioles in the early stages of the transformation; in both the axial filament originates from one of the centrioles; the caudal migration and final disappearance of a granulelike structure in close association with the axial filament take place in both. They are also similar in the appearance of four centrioles just as the spermatid nucleus begins to elongate and in the participation of the centrioles in the formation of the middle piece. Otte's recognition of the granulelike structure, which migrates caudad and finally disappears, is significant. This structure he interprets to be the entire distal centriole, while we believe it is only a derivative of the distal centriole. Recently Johnson (1931) finds in *Locusta viridissima,* the species studied by Otte, that it is not the entire distal centriole which migrates caudad as Otte thought, but only a derivative of this. This being the case, the four centrioles, which Otte believed to have arisen from the proximal centriole, must have come from the division of the original centriole into proximal and distal ones, each of which in turn divides into two, giving rise to four, as in *Amblycorypha.* His description of the migration of two centrioles up into the nucleus, their subsequent division into four, and their final fate to form the "Innenkörper" in the sperm nucleus, we cannot correlate with our observations. These four centrioles in the nucleus of the older spermatids in *Locusta* look more like some basichromatin granules in the center of many clear areas in the nucleus of the spermatid in *Amblycorypha.*

The behavior of the centrioles in *Steiroxys,* as observed by Davis (1908), is almost identical with that of *Amblycorypha.* However, Davis seems to have overlooked the presence of centrioles in the young spermatids. It is only when the nucleus begins to elongate that he notes the presence of these structures. Hence he does not determine the origin of the axial filament nor does he notice the presence of a granulelike structure lying on it or in close association with it. It seems probable that, if a further study of the centrioles in *Steiroxys* and *Locusta* were

made, a condition much more like that in *Amblycorypha* might be found.

SUMMARY

1. The mass of chromosomes in each daughter cell after the last maturation division breaks up into patches, which gradually become diffused through the nucleus. Later a differentiation of the nuclear material into a basichromatin substance and an oxyphilic substance takes place. From the basichromatin substance is formed the solid, deeply staining, fusiform sperm nucleus.

2. The mitochondrial substance assorted to each spermatid clumps into a mass. This mass rapidly condenses and finally rounds out to form the spherical nebenkern. The nebenkern, after undergoing many changes, differentiates into an inner chromophilic core and an outer chromophobic part, surrounded by a well-marked membrane. Later it comes to lie on or below the axial filament, elongates with this structure, and finally forms a covering around the flagellum.

3. The dictysomes in each spermatid fuse. The mass resulting from this fusion comes to lie against the nuclear membrane near the nebenkern. From it a vesicle is differentiated which increases rapidly in size to form the acroblast. The acroblast migrates around the nucleus, and, after the formation of the acrosome complex in close association with it, it is finally cast off into the cytoplasm. The acrosome complex, which consists of a vesicle containing a deeply staining granule, becomes the definitive acrosome, having the shape of an inverted V.

4. The spherical centriole divides into proximal and distal parts. From the distal centriole a derivative arises, which migrates caudad and finally disappears from view in the last stages of the transformation. Later the proximal and distal centrioles divide. The four centrioles resulting from this division participate in the formation of the middle piece.

5. The axial filament apparently originates from the central bodies, but it has not been determined whether or not it originates from the distal centriole. From it the comparatively short flagellum of the mature spermatozoön is formed.

6. The spermatozoön consists of a large inverted V-shaped acrosome, which stains lightly or not at all; a solid, deeply staining, fusiform nucleus, to the posterior end of which is attached the scarcely distinguishable compact middle piece; and

a short flagellum, which measures approximately twice that of the head.

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ILLUSTRATIONS

[All figures were drawn with a camera lucida at an initial magnification of 2,700, and the drawings retouched freehand.]

AMBLYCORYPHA OBLONGIFOLIA (DE GEER)

PLATE 1

FIGS. 1 to 3. Anaphases of the second maturation division. Mitochondria near the cell wall and dictyosomes scattered along the spindle plate.

FIG. 4. Cross section of cytoplasm at the stage shown in fig. 3, showing the distribution of mitochondria.

5. Late anaphase; showing short and long mitochondrial threads, also the small dictyosomes at both ends of the disappearing spindle fibers.

6. Telophase; the number of long mitochondrial threads increases.

7. Cross section of cytoplasm at the stage shown in fig. 6, showing the distribution of mitochondria.

8. Telophase.

FIGS. 9 to 14. Successive stages in the condensation of mitochondrial substance to form the nebenkern. Note also the dictyosomes.

FIG. 15. Early spermatid; showing nebenkern, axial filament, and small vesicle differentiating from the dictyosome mass.

FIGS. 16 and 17. Two spermatids with fully formed acroblast; nebenkern differentiating into two substances.

18 and 19. Spermatids at a little later stage; centriole divides into proximal and distal parts. Note centriole derivative a short distance from the distal centriole.

20 to 24. Later spermatids; nebenkern starts elongating; centriole derivative at its posterior end; acroblast moving around the nucleus and increasing in size.

PLATE 2

FIG. 25. Late spermatid; dictyosome mass becomes diffused from which acroblast begins to separate.

FIGS. 26 to 29. Stages in the formation of acrosome complex.

30 to 33. Note increase in size of acrosome complex.

FIG. 34. Elongation of nucleus; flattening of acrosome complex.

FIGS. 35 and 36. Later stages. Note caplike acrosome and paddle-shaped nucleus and division of centrioles.

37 to 39. Slightly later stages.

FIG. 40. Note nucleus and acrosome; basichromatin of nucleus becoming compact.

FIGS. 41 and 42. Cross sections of flagellum and nucleus, respectively, at the stage shown in fig. 40.

FIG. 43. Very late stage in the transformation. Note change in staining reaction of acrosome.

44. Nearly mature spermatozoön; acrosome stains lightly and uniformly; nucleus very compact except at anterior portion. Note protoplasmic ball at posterior end of flagellum and also undulating membrane on each side of the nucleus.

45. Side view of spermatozoön at the stage shown in fig. 44.

46. Cross section of solid nucleus.

47. Mature spermatozoön.

FIGS. 48 and 49. Cross sections of nucleus and flagellum, respectively, of mature spermatozoön.

PLATE 1.

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PLATE 2.

FAUNA PHILIPPINENSIS (PLATYPODIDÆ ET SCOLYTIDÆ), III

By KARL E. SCHEDL

Of Vienna, Austria

PLATYPUS PHILIPPINENSIS Blandf.

Female.—Nearly black, 6.20 mm long, 4 times as long as wide.

Front flat, epistomal margin and the median line up to the center shining, polished, and with a few small punctures; anterior half densely punctured, subopaque and areolate above.

Pronotum shining, 1.29 times as long as wide, widest behind the lateral emarginations; the latter shallow; median sulcus long and fine, surrounded by cordiform patch of densely placed and uniform-sized punctures, the patch longer than wide; surface with small, sparingly placed punctures.

Elytra but little wider than the pronotum (16 : 15.5) and 2.31 times as long, sides parallel, rather narrowly rounded behind; striate-punctate, with the exception of the eighth all striæ impressed, punctures of the first and ninth striæ confluent on the greater part of their length, striæ two to seven with rather large and deep punctures, the eighth striæ with the punctures small, shallow and remotely placed, interstices subconvex and impunctate, base of the third widened and with a long row of densely placed transverse rugæ; declivity rugose and hairy, convex above, perpendicular below. Abdomen normal.

Types in the possession of Mr. F. C. Hadden and in my own collection. The female is here described for the first time.

Locality.—Mount Maquiling, Laguna Province, Philippine Islands (*Bautista*). Under bark of malugai, *Pometia pinnata* Forst.

PLATYPUS HYBRIDUS sp. nov.

Male.—Nearly black, 6.30 mm long, 3.04 times as long as wide. A very distinct species of the group *Platypi sulcati.*

Front opaque, depressed medially, densely areolate; antennal scape longer than wide.

Pronotum quadrate, shining, widest behind the moderately deep lateral emarginations, coarsely moderately densely punc-

tured; median sulcus fine and long, in front of the sulcus surface impunctate.

Elytra as wide and 1.95 times as long as the pronotum, sides parallel, broadly rounded behind, striate-punctate, striæ very narrow and very deeply impressed on the anterior two-thirds, becoming shallower to obsolescent towards the apex, interstices convex near the base, gradually flattening out caudad; in front of the first four interstices a narrow transverse area covered with transverse rugæ, the interstices shining and sparsely punctured on the basal fourth of their entire length, opaque and rather densely covered with irregularly placed, deep, shining punctures behind, the punctures becoming more numerous and shallower towards the apex, with rows of reddish hairs; cylindrical, declivity convex and flattened on an equilateral triangular areas as in some species of *Xyleborus*, near the upper corner with a granule in the prolongation of the second interstice; a few more but smaller granules on the flattened portion below. Abdomen normal.

Type in my collection.

Locality.—Philippine Islands. Collected from anang, *Diospyros ahernii* Merrill.

PLATYPUS SPECTABILIS sp. nov.

Female.—Dark reddish brown, the anterior half of the elytra paler, 6 mm long, 2.95 times as long as wide. This species shows characters of the pronotum that have not been known in other species of the family. Preliminarily I place it in the *Platypi sulcati.*

Front plano-concave, depressed in the center, subopaque, subimpunctate on the anterior third, areolate above, lateral margin towards the articulation of the antennæ raised and longitudinally wrinkled. Antennal scape longer than wide.

Pronotum quadrate, lateral emarginations weakly developed, surface shining and with scattered fine punctures; median sulcus long, extending nearly to the middle of the pronotum, with a cordiform patch of rather large, deep punctures around it; the patch much longer than wide, transverse in front, anterior to this patch with a group of nine to twelve large pores on each side of the median line, the outer line of these pores is semicircular on one side, nearly quadrate on the other.

Elytra little wider than the pronotum (21 : 19.5) and 2.05 times as long, the base feebly carinate, sides subparallel, broadly rounded behind; surface shining on the anterior two-thirds,

subshining behind; sulcate, sulci narrow, irregularly uniseriately punctured, disappearing towards the declivity; interstices convex on the shining portion, flat behind, with fine, widely scattered punctures; the ninth interspace densely, roughly punctured, base of interstices two to five transversely rugose, more strongly on the third; declivity commencing behind the anterior two-thirds, feebly convex at first, perpendicular behind, the perpendicular face triangular, the entire declivity covered with extremely crowded small shining punctures on an opaque surface and with numerous short yellow hairs. Abdomen normal.

Type in my collection.

Locality.—Philippine Islands. From lumbayao, *Tarrietia javanica* Blume.

CROSSOTARSUS SUBDEPRESSUS sp. nov.

Male.—Dark reddish brown, declivity black, 4.56 mm long, 3.13 times as long as wide. Belonging to the group *Crossotarsi subdepressi* this species is easily recognized by the noncarinate interstices on the upper convexity of the declivity and the characters of the last abdominal sternite. The latter is opaque and has a rather deep emargination on the caudal margin, thus forming a lateral bluntly rounded edge on each side.

Front narrowly depressed above the epistomal margin, convex above, entirely opaque, with a small shining puncture in the center and a few scattered punctures towards the vertex.

Pronotum brightly shining, slightly longer than wide 15 : 14), widest behind the rather shallow lateral emarginations; median sulcus weakly developed, with scattered fine punctures; a few coarser ones at both sides of the median line in front of the sulcus, densely but finely punctured on a narrow strip along the basal margin.

Elytra slightly wider than the pronotum (15 : 14) and 1.80 times as long, widest a short distance behind the middle, sides subparallel, broadly rounded and with a semicircular emargination behind; base carinate, surface shining, striate-punctate, striæ impressed, less so laterad, strial punctures small and confluent on the first five striæ, punctures little more remotely placed at the sides, interspaces flat, with scattered fine punctures on the disc, punctures becoming very densely placed towards the base and the sides; declivity commencing in the posterior third, uniformly strongly convex, with a lunate nearly perpendicular depression below; the shining interstices, which are of equal width, cease suddenly but without any armature

at the upper limit of the declivity; the latter opaque with rows of yellow hairs, the upper limit of the lunate depression developed as a feebly raised line; the lateral processes with one or two small spinules on the outer margin.

Female.—Reddish brown, pronotum and basal part of the elytra paler, 4.75 mm long, 3.37 times as long as wide.

Front flat, with a hump-shaped elevation in the center; surface opaque, with few scattered punctures in front and shallow strigose punctures behind; angle separating the front from the vertex acute.

Pronotum longer than wide (15.5 : 13.5), lateral emarginations shallow, surface shining, minutely reticulate, and with widely scattered punctures; median sulcus fine and long, surrounded by an oval patch of densely placed punctures, with a depression on each side of the median line in front of the sulcus.

Elytra wider than pronotum (14.5 : 13.5) and twice as long, sides parallel, with a lunate emargination behind; sculpture as in the male but the punctuation not so deep; the lateral processes shorter and simple. Abdomen normal.

Types in the possession of Mr. F. C. Hadden and in my collection.

Localities.—Mount Maquiling, Laguna Province, and Quezon Park, Tayabas Province, Luzon, Philippine Islands; F. C. Hadden, collector. From *Ficus* sp., and hagimit, *Ficus minnahassae* (Teysm. and De Vr.) Miq.

CROSSOTARSUS OCTOCOSTATUS sp. nov.

Male.—Reddish brown, declivity darker, 3.88 mm long, 3.33 times as long as wide. A member of the group *Crossotarsi subdepressi* with the interstices 1, 3, 5, 7 costate on the upper convexity of the declivity.

Front flat, shining, subimpunctate below, rugosely and longitudinally punctured above, with an elevated carina from the center towards the vertex, interrupted for a short piece above the center, upper surface rather densely hairy.

Pronotum longer than wide (38 : 33), lateral emarginations shallow; median sulcus short and very fine; surface shining, finely punctured; punctures rather densely placed near the apical margin, scattered and inconspicuous behind.

Elytra wider than pronotum (36 : 33) and 1.78 times as long, sides subparallel, broadly emarginate behind; disc very finely lineate-punctate, punctures hardly visible at the sides; interstices flat and impunctate, somewhat convex near the base where the

third is conjoined with the first and fifth, base of the third without any remarkable punctuation; declivity commencing in the posterior third, convex above, perpendicular and with a lunate depression below, the lateral processes bent backwards and downwards, the interstices 1, 3, 5, 7 costate on the anterior half of the upper convexity, nearly horizontal and then abruptly ceasing, the other interstices low, ending at the commencement of the carinæ, the rest of the convexity opaque, all interstices continued as low ridges which are covered with a row of setose granules, the first spined at the upper border of the shining and impunctate lunate depression, the lower margin of the latter with a small tooth between the suture and the lateral process, the process with a slender spine at its apex. Abdomen normal.

Female.—Colored as the male, 3.97 mm long, 3.26 times as long as wide.

Front as in the male but a little more convex, punctuation and median carinæ finer.

Pronotum subquadrate (37 : 35) but of the same sculpture as in the male.

Elytra but little wider than pronotum (36 : 35) and 1.80 times as long, sides parallel on the anterior half, narrowed behind, transverse at the apex; disc with sculpture similar to that in the male but still finer; the base of the third interstice with a few minute granules; declivity opaque, densely hairy and feebly convex above, with a shining, lunate, perpendicular depression below; lateral processes blunt. Abdomen normal, convex.

Types in the possession of Mr. F. C. Hadden and in my collection.

Locality.—Mount Maquiling, Laguna Province, Philippine Islands; F. C. Hadden, collector.

CROSSOTARSUS CONCAVIFRONS sp. nov.

Female.—Reddish brown, 4.17 mm long, 3.30 times as long as wide. A remarkable form, which is easily recognized by the characters of the front. I cannot place it in any group until the other sex is known.

Front with a transverse, deep impression behind a narrow epistomal margin, the concavity extending up to the lower margin of the eyes at the sides, much less so in the center; a plush of long erect hairs on each side of the epistomal margin shortly in front of the antennal articulation; the concavity shining, the upper half of the front convex and densely covered with very long, erect, yellow hairs, abraded in one of the specimens; an-

tennal scape about as long as wide, with a fringe of hairs on its outer margin, the longest of which are three times as long as the scape.

Pronotum subquadrate (39 : 37), rather feebly convex, lateral emarginations rather shallow, surface shining, with three more or less distinct depressions on each side of the median line, median sulcus fine, surrounded by a circular patch of densely placed, fine punctures which rather gradually become coarser and less densely placed at the extreme margin; the remaining surface rather coarsely and moderately densely punctured and with a pubescence that is denser than in most Platypodidæ and resembles that of *Cenocephalus pusillus* Schedl.

Elytra wider than pronotum (40 : 37) and 1.79 times as long; base finely carinate, sides parallel on the anterior two-thirds, narrowed along the upper convexity of the declivity, transverse behind; striate-sulcate, sulci indistinctly multipunctate; interstices convex, the first narrow and with a row of tubercles on its entire length, the others with sparsely placed fine punctures, the punctures coarser and denser on the second, base of the third with a long row of crowded, round, fine granules; declivity convex, with granulate interspaces; shallow sulci and reddish pubescence above, with a perpendicular finely rugose face below, a plush of reddish hairs on the latter. Abdomen normal.

Types in the Zoologisches Museum in Hamburg and in my collection.

Locality.—Philippine Islands.

CROSSOTARSUS COLEOPTRATUS sp. nov.

Male.—Black, 4.75 mm long, 3.37 times as long as wide. The third male known of the group *Platypi coleoptrati.*

Front flat, hardly visibly impressed below, subshining, punctured on a triangular space below, coarsely, longitudinally wrinkled above, with a short, median, impressed striga.

Pronotum 1.23 times as long as wide, shining, with a very long median sulcus, which is continued anteriorly by an impressed line, coarsely uniformly punctured all over.

Elytra wider than pronotum (14.5 : 13.2) and 1.84 times as long, base carinate, disc striate-sulcate, sulci narrow, indistinctly punctured, all interstices equal, strongly convex, narrow, subimpunctate; the ninth interstice densely irregularly punctured, near the base the sulci but little wider and more distinctly and densely punctured; declivity rather steep, truncate, subconvex, with three processes on each side, the apices of the median lower pair as widely separated from each other as the first process

from the second, the third much closer to the second and formed by the carinate ninth interstice which terminates abruptly at the lateral margin of the declivital convexity, the first and fourth interstices decreasing in height and width towards the upper declivital margin, the others carinate and terminating abruptly at the commencement of the truncate declivity, the latter shining and the interstices continued as rows of coarse granules. Abdomen normal.

Type in my collection.

Locality.—Philippine Islands.

STENOPLATYPUS ORNATICEPS sp. nov.

Male.—Nearly black, 4.26 mm long, 3.52 times as long as wide. The only relative of this species known may be *C. taiheizanensis* Mur., from Formosa, which must be transferred to *Stenoplatypus* Strohm. It differs from the latter by the presence of spines on the abdominal sternites and in the armature of the declivity.

Front feebly convex, subopaque, with a few round punctures in front, longitudinally wrinkled behind.

Pronotum slightly longer than wide (38 : 35), shining, median sulcus hardly visible, with very fine and very sparsely placed punctures.

Elytra wider than pronotum (37 : 35) and 1.86 times as long, striate-punctate, sutural striæ impressed, others near the base and apex only; strial punctures indistinct to obsolescent; interstices convex towards the base and apex, with scattered very fine punctures, not remarkably stronger near the base; declivital armature similar to that in *C. taiheizanensis,* but the fused first and second interstices forming a long blunt tooth, third and fourth subequal in length, the third broad and blunt, the fourth very narrow and without apical tooth; the lateral process consisting of a large blunt external and two small inner teeth; the apical margin without armature; the third and fourth visible abdominal sternites with two widely separated pointed teeth, those of the fourth sternite being much larger.

Female.—Nearly black, 4.36 mm long, 3.46 times as long as wide. A single specimen from an old collection and also originating from Luzon, Philippine Islands, I am inclined to regard as the other sex.

Front as in the male but with the sculpture shallower.

Pronotum little longer than wide (40 : 37), shining, with a long, strongly impressed, median sulcus and with three large pores, which are situated in an equilateral triangle at each side

of the sulcus, surface with widely scattered and very fine punctures, a small group of more densely placed punctures in front of the pores.

Elytra wider (40 : 37) and 1.87 times as long as the pronotum, sides parallel, narrowed along the declivital convexity, transverse at the apex; sculpture similar to that in the male, the striæ becoming obsolescent behind; the base of the third interstice densely punctured and with some indications of low transverse ridges; declivity convex above, perpendicular below, rugose and with short yellow pubescence. Abdominal sternites convex and unarmed.

Types in the possession of Mr. F. C. Hadden and in my collection.

Localities.—Males, Mount Maquiling, Laguna, Luzon, Philippine Islands, F. C. Hadden, collector; female, Luzon.

XYLEBORUS PERPILOSELLUS sp. nov.

Female.—Reddish brown, 2.37 mm long, twice as long as wide; closely allied to *X. punctulatus* Egg. but stouter and much more densely pubescent.

Front opaque, convex, indistinctly punctured except on the epistomal margin where the punctures are deeper, finer, and more densely placed. Eyes strongly emarginate in front; antennal club distinctly wider than long.

Pronotum globose, 1.26 times as wide as long, base transverse, posterolateral angles not rounded, rectangular; sides parallel on the posterior third, evenly broadly rounded in front; anterior margin not armed; summit behind the middle, anterior area finely asperate, posterior area subshining, minutely reticulate and densely finely punctured.

Elytra wider than pronotum (39.5 : 38) and 1.56 times as long, sides parallel, broadly rounded behind, convex from the base to the apex, apical margin not acute but rounded; the entire surface densely, finely, and irregularly punctured and with a fine, dense, yellow pubescence.

Types in the possession of Mr. F. C. Hadden and in my collection.

Locality.—Mount Maquiling, Laguna Province, Luzon, Philippine Islands, F. C. Hadden, collector.

XYLEBORUS FASTIGATUS sp. nov.

Female.—Reddish brown, elytra darker, 2.72 mm long, 3.34 times as long as wide. A small species of the general shape of *X. amphicranoides* Hag.

Front subshining, plano-convex, with a narrow, shallow, transverse depression between the eyes, which is interrupted by a low longitudinal elevation, finely, deeply, and densely punctured on the epistomal margin and towards the eyes, impunctate along the median line, more sparsely and shallowly punctured above.

Pronotum shining, 1.20 times as long as wide, base transverse, posterolateral angles rounded, sides parallel on the anterior two-thirds, rather broadly rounded in front; summit before the middle, anterior area densely finely asperate, polished, finely and moderately densely punctured behind, impunctate along the median line.

Elytra as wide and 1.75 times as long as the pronotum, sides parallel on more than the anterior three-fifths, then rather obliquely narrowed, deeply emarginate at the suture; disc shining, lineate-punctate, striæ not impressed, consisting of regularly placed moderately fine punctures, those of the sutural striæ somewhat coarser, interspaces flat and covered with an irregular row of finer punctures; declivity commencing at the middle, concave as in *X. amphicranoides,* concavity subshining, subimpunctate, the suture elevated, lateral margin armed with two large slender spines, which are directed backwards, the first pair situated at the anterior fourth, the second pair at the middle of the declivity; second pair but little larger than the first, cephalad to the first spine with a pair of small sutural teeth and two pairs of small setose tubercles; the apical emargination deeper than wide; the lateral processes with a small tooth, which is directed inwards on the inner margin, the outer one with a row of setose tubercles, the apices pointed.

Types in the possession of Mr. F. C. Hadden and in my collection. The specimens bear the label: Acc. No. 195, Sch. of For. Univ. P. I.